

## INTERVIEW

# The people behind the papers – Peter Kozulin, Rodrigo Suárez, Qiong-Yi Zhao, Linda Richards and Laura Fenlon

The neocortex is unique to mammals and so, for evolutionary studies, researchers have compared eutherians and marsupials. A new paper in *Development* uncovers key differences in the timing of gene expression changes in the cortical development of the mouse and the similarly sized marsupial, the fat-tailed dunnart. We caught up with the authors from The University of Queensland, Australia, to find out more about their research and their future plans.

## Laura, Linda and Rodrigo, can you give us your scientific biographies and the questions your labs are trying to answer?

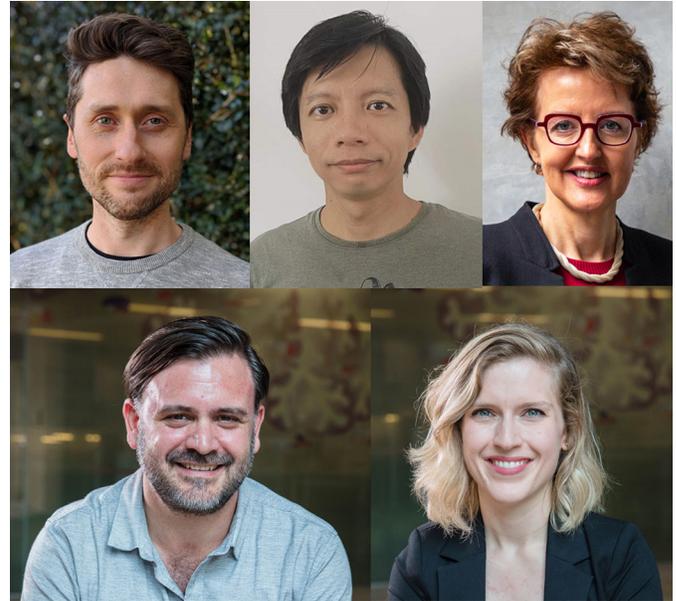
**LF:** I completed a PhD and short postdoc under the supervision of Linda at the Queensland Brain Institute, The University of Queensland, working on the development and plasticity of the neocortex and its connections. In 2021, I took up a faculty position leading my own research group at The School of Biomedical Sciences, The University of Queensland. I am now investigating the development and evolution of the brain, continuing to use marsupials as an example of evolutionary divergence. In particular, I am now focusing on how the interactions between the environment and gene expression can affect the neocortex between species and across development.

**LR:** Our lab recently moved to St. Louis, USA, and we have established a colony of fat-tailed dunnarts to study early brain development. We are interested in how circuits are first established, and molecular and activity-dependent mechanisms that regulate their development.

**RS:** I studied Biology at the University of Chile, where I developed a career-defining interest about the self-assembling properties of living systems. My PhD, under the supervision of Prof. Jorge Mpodozis, studied the evolution of pheromone-sensing systems across mammalian lineages, including the role of ecological contexts in shaping ontogenetic and phylogenetic plasticity. In 2011, I joined Linda's group at the Queensland Brain Institute as a postdoctoral fellow, where I focussed on the development and evolution of the cerebral cortex. I was then recruited by the School of Biomedical Sciences, The University of Queensland, where I lead the Brain Evo-Devo group. My main research questions are: how can changes in developmental processes generate evolutionary innovations, and how do nature and nurture interact in the generation of complex phenotypes?

## Peter and Qiong-Yi, how did you come to work on this project and what drives your research today?

**PK:** This was one of the first projects I worked on when joining the marsupial group as a postdoc at the University of Queensland, and I have been a part of every stage of its progress since its conception.



Clockwise from top left: Peter Kozulin, Qiong-Yi Zhao, Linda Richards, Laura Fenlon and Rodrigo Suárez.

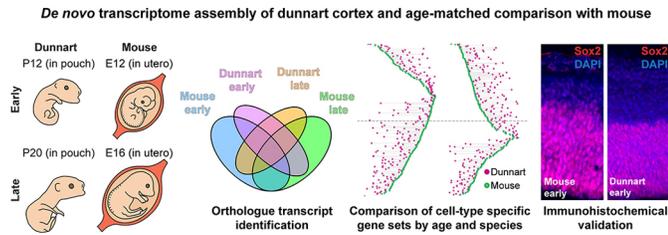
Throughout my career I have investigated the molecular processes underlying the development and evolution of the mammalian nervous system, and I was excited to join the dunnart project as it allowed me to focus on the neocortex, a brain area I had not previously studied in detail. The results were incredibly interesting and have motivated me to continue working with the dunnart as a fantastic model of mammalian cortical development. We have only begun to scratch the surface of the insights this animal model can offer.

**Q-YZ:** There are many anatomical differences between the brains of eutherians and marsupials, which introduces interesting evo-devo questions between the lineages. Therefore, we proposed to investigate the transcriptome-wide difference between mouse and fat-tailed dunnart. As a bioinformatics researcher, I took this opportunity to dive into the transcriptomic world of marsupials and eutherians, along with a wonderful collaboration journey with my co-authors.

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

**PK, RS, Q-YZ, LR & LF:** Only mammals evolved a six-layered cerebral cortex, and within mammals, only eutherians (aka placentals) evolved a corpus callosum. In previous research we discovered a remarkable conservation of cortical connectivity across species, and that master regulatory genes differed in the general timing of expression. In this study, we examined the genetic makeup of marsupials and placentals of similar size (dunnarts and mice), at stages equivalent to deeper- and upper-layer neurogenesis to compare their maturational states. We found that the marsupial cortex exhibits markers of mature neural systems earlier than mice, and that at later stages these become less distinct. Such a pattern is consistent with their different reproductive strategies and likely

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### Cortical development in mouse and dunnart.

reflects differences in early behaviours (e.g. marsupials need to find the teat before cortical development), which in turn might help us better understand the contexts in which key innovations in cortical development originated, more than 100 million years ago.

### How did you start working on the fat-tailed dunnart?

**LR:** Marsupials provide an ideal model to investigate early brain development and to conduct evolutionary comparisons to gain insights into fundamental principles. I studied marsupial visual system development in the lab of Dr John Nelson at Monash University in Melbourne when I was an undergraduate student. We worked with over 100 different species of marsupials in John's lab in a large comparative project and I was fascinated by their neuroanatomy. I knew that marsupials would provide an excellent model for comparative studies on early brain development, but we did not have the molecular tools at the time. Later, when Rodrigo joined my lab we looked for a marsupial species that we could easily breed in captivity and establish a colony. Fat-tailed dunnarts were ideal because of their small size (around the size of a mouse) and quick breeding cycles. As they are born at the equivalent of an embryonic day 10 mouse, much of their brain development occurs in the pouch.

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

**PK:** As many researchers involved in bioinformatics work know, it can be easy to get lost in the high volume of RNA sequence data. As I performed multiple data analyses, the picture of differential molecular cortical development across species slowly emerged and so it is difficult to pinpoint a precise eureka moment. However, the time when I generated the first principal component analysis of the age-matched cortical datasets with the ENCODE developmental series stands out in my memory as it really validated the initial results of a different rate of cortical development across species.

**RS:** Things that were pleasant findings included the remarkable conservation of transcripts between species and ages, which indicated that presence (or absence) of gene products alone was not likely to be the main explanation for phenotypic differences and also the consistent finding that equivalent early stages of cortical development showed more mature profiles in marsupials than eutherians.

**Q-YZ:** *De novo* transcriptome assembly for the fat-tailed dunnart was challenging due to the high computational requirements. I was really happy and relieved when we finally got the reconstructed transcript sequences after many attempts. This was the first dunnart transcriptome in the world! It was particularly interesting when we further identified a group of candidate genes, the expression profile of which showed dramatic differences between mouse and fat-tailed dunnart via the ranking analysis.

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

**PK:** I took a long time to complete some of the bioinformatics analyses, partly because I was studying for a Master of Bioinformatics course to better my skills for projects such as this. Thus, I was not undertaking my postdoc work full time. However, my work on this project was certainly helped by this additional training and I am extremely happy with the final product!

**RS:** Rather than frustration, it was difficult to work with a poorly studied species (at least compared with mice), which we had to start breeding from scratch. This was particularly challenging from a bioinformatics perspective, as no annotated genome has been published.

**Q-YZ:** I was a bit frustrated after a couple of fails for the *de novo* transcriptome assembly task. With the help of our IT supporting team, we solved issues and finally made it.

### Do you think that the differences in timing of gene expression will explain the difference in the structures connecting the hemispheres of the neocortex in marsupials and eutherians?

**PK, RS, Q-YZ, LR & LF:** Certainly, this is a hypothesis that we consider possible and indeed we have demonstrated in a previous publication (Paolino et al., 2020) that manipulating the timing of expression of just one transcription factor (SATB2) is sufficient to change the connectivity pathways in both mouse and dunnart. The results from the article presented in this issue of *Development* suggest that many more genes may be involved in the timing differences driving the different connectivity of marsupial and eutherian brains, and we are excited to use this foundation to continue to uncover what these might be and how they are coordinated in the future.

### Do you think temporal regulation of gene expression could be sufficient to explain the relative expansion of the neocortex in humans versus mice?

**PK, RS, Q-YZ, LR & LF:** This is a long-standing question in the field of evolutionary biology, especially because the extraordinarily protracted development of the human brain compared with that of mice is one of the main distinguishing features during development. Timing is certainly a crucial factor, but also the relative matches and mismatches in timing of transcriptional networks, and therefore the processes that these networks coordinate. For example, the absolute length in days that neuronal migration takes may be a significant factor driving interspecies brain diversity, but also the extent to which neuronal migration temporally overlaps with the processes of subsequent neuronal birth or axon extension and synaptic connectivity may be an equally important factor.

### What is next for you after this paper?

**PK:** Rodrigo and Laura recently set up their independent labs last year at the School of Biomedical Sciences in The University of

Queensland and I was lucky enough to be invited to join their new group. I am excited to continue to use the dunnart model to study other aspects of mammalian cortical evolution such as identifying molecular factors that determine projection neuron identity. I am also interested in broader aspects of molecular evolution in vertebrates, and I would like to begin to undertake more high-throughput bioinformatics projects across multiple clades of species and integrated data types, e.g. genomic, epigenomic and RNA, in pursuit of this goal.

**Q-YZ:** As I lead the bioinformatics core at Queensland Brain Institute, I will continue to support ongoing research projects by advising on experimental design and ensuring timely and accurate analysis of next-generation sequencing data. Although my role provides an analytical service for research groups in the institute, many projects require bespoke programming and analysis, allowing me to make important intellectual contributions to individual projects. This paper shows a good example.

#### **Where will this story take your labs next?**

**LF:** We hope to build on these findings to understand the mechanisms that drive the altered time course between species across evolution, as well as the consequences of heterochronic events on the final outcome of the brain.

**RS:** This study opens many new research avenues. For example, a better understanding of the genes involved in cortical development offers clues on regulatory networks essential for healthy formation of the human brain, as well as how slight modifications on these can lead to disease, at the ontogeny level, or to evolutionary change at the phylogeny level.

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

**LF:** I love to cook and learn about food from all over the world. I find that it's a great parallel to a career in scientific research because you can do research on existing recipes, formulate hypotheses (for example about how to improve a dish), test those hypotheses with an experiment and then (hopefully) enjoy the results!

**LR:** In my spare time I enjoy hiking, reading crime novels, working out, cooking and spending time with friends and family.

**RS:** There are some of the world's most spectacular beaches within an arm's reach of Brisbane for a nice day out. Something that I particularly enjoy is scuba diving, as the underwater landscape is breath-taking and you can easily encounter turtles, manta rays, dolphins and friendly sharks, amongst a panoply of reef fish, corals and brightly coloured nudibranchs.

**PK:** I love spending all my spare time with my family, especially since my wife and I had our first baby this year!

**Q-YZ:** I like a lot of activities in my spare time, such as playing table tennis, swimming, mountain climbing, etc. I also enjoy playing video games with my kids, especially when we are 'locked' at home during the Covid pandemic.

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