Molecular Evolution in Copenhagen

The Sources of Complexity

Peter Arctander was an ornithologist when he began to explore molecular ways of reconstructing phylogeny. In his population studies he later took tissue samples from more safari animals than a big game hunter could dream of shooting in a lifetime. Now he's changed direction again, plunging into the wonders of alternative splicing and non-coding RNAs.

Evolutionary biologist Peter Arctander likes research fields where methodology is not yet understood, where major questions remain unanswered and where testing a hypothesis is a leap in the dark. When he graduated as a traditional biologist in Copenhagen 1988 the molecular reconstruction of phylogeny was just such a field. DNA promised to yield much more information than morphological traits and a spell at Allan C. Wilson's lab at Berkeley was seen almost as a rite of passage for anyone wishing to do empirical work in the field of molecular evolution.

It was the good old days, when PCR meant carrying tubes by hand from one water bath to another. "And Allan Wilson had great inspirational power. He was not only knowledgeable and smart, but also extremely enthusiastic for the fundamental understanding of evolution and how to find out about it." What Arctander is still most impressed about is Wilson's creativity: "He had one good idea per day. Maybe only 5% of them were *really* good ideas, but most scientists have only one or two good ideas a lifetime! He was a nuclear explosion of good ideas."

Peter Arctander (front) and his current team

Mitochondrial DNA proved to be as useful in Arctander's study of perching bird evolution as Wilson's lab was inspiring. So for some years Arctander explored the opportunities and pitfalls of molecular phylogenetics, reconstructing branches in the tree of life, trying to establish the limits of resolution. Copenhagen University still remains the proud owner of the world's largest collection of bird blood samples, assembled at that time.

Arctander was fascinated by technology and by the uses that it might have in conservational biology and in the study of more recent development of species on the population level. This idea inspired a huge project in Uganda, the African Wildlife Genetics Program, for which he organised financial support from Danish development agencies (DANIDA). He wanted to develop local expertise in molecular evolutionary biology as a way of supporting conservation biology. "This is important also from an economic viewpoint, because half of gross national product in Uganda is wildlife-related tourism."

Arctander's group helped to build a state-of-the-art molecular biology lab at Makerere University in Uganda's capital, Kampala, which opened in 1999. The project remains a great success for its study of recent speciation processes and understanding variation in populations. More than 20 species of the large herbivores south of the Sahara have been analysed. Tissue samples were collected from thousands of individuals. "I 'shot' more safari animals for tissue samples than any big game hunter could dream of shooting in his lifetime", Arctander says, laughing. Due to ill health, he has now left the project. It has been continued by his colleagues in Copenhagen and Kampala.

"Playboy biology"

Judging from his publication list, it seems that little in molecular evolution has escaped Arctander's interest. He does claim to have had some 'no-goes', although these were sometimes hard to defend. "I had two brilliant students that wanted to do something with ancient DNA of humans, but I said: 'No, I'm not doing research on humans. I'm not going to supervise you.' However, they were very pushy, and came again and again asking what they could do with ancient DNA, which I didn't want to do either."

Arctander: "So in the end I said: 'There's only one thing I would like to do and that is examine the Greenland deep ice core samples, looking for whatever old DNA could have been preserved there. Look at it the same way as pollen in soil and lake drillings as well as studying how DNA degrades over time in an ice-environment.'" He laughs: "Maybe I liked that because it's playboy biology, like tissue sampling live elephants in Africa". They used PCR to amplify fragments of well-characterized genes in ice cores dated to 2000 and 4000 years ago. After sequencing the recovered fragments, they compared them to known sequences in a database and found that the ice cores held the remains of a surprising diversity of life forms. One of the insistent students who sparked this project, Eske Willerslev, has since become Professor for Ancient DNA at the University of Copenhagen.

"Something is missing in pure Darwinism"

In the last ten years Arctander has developed an interest in a topic he calls the evolution of complexity. Using molecular data for reconstructing trees and studying variation in populations holds little information on the evolution of basic biological processes. "I studied the history of evolution, what paths evolution took, but there's this one question in my mind that has been there a long while", he says. "What is the source of organismic complexity?" More than ever, he is astonished by the incredible amount of information that just one cell has to manage and integrate, all the molecular processes it has to carry out, to send, receive and store messages. "And we consist of billions of cells! We know very little of the molecular complexity that underlies the complexity in multicellular organisms."

"What happened 900 million years ago, when organism complexity took a great leap? Maybe there is one single origin for this complexity." For him, the protein-centred view of evolution has led research astray. He explains: "Exons make up only 1.8 % of all human DNA and this doesn't correspond to our level of organismic complexity". He is irritated by the textbook assumption that evolution proceeded and new organisms emerged through selection of mutations in the protein-coding DNA. To him that isn't very convincing.

"Creationists argue that today's mainstream Darwinism does not properly account for the evolution of life's complexity and diversity. With this I tend to agree. Don't get me wrong, I'm a selectionist, a Darwinist, but still I think that our theories don't answer the question of complexity properly. We are missing something." So in the Danish evolution-creation debate he argues that the creationist explanation for complexity appears as valid as the evolutionary one. Obviously, this provokes disapproval from some colleagues. Instead of making this a battle between religion and science, evolutionary biology should come to the debate better prepared, with persuasive evidence to fill the gaps in its explanations.

Splicing is astonishingly conserved

Arctander has long suspected that the source of this complexity lies not within exons but outside of them, in regulatory RNA. His suspicion is fed by the fact that more than 50 % of the genome is transcribed to RNA and only 1.8 % translated to proteins. "No one was interested when we first started looking at introns and non-protein coding RNA," Arctander says. Very few people think along these lines. One of them is John S. Mattick from the University of Queensland in Australia. After collaboration, they showed in 2000 that the number of alternatively spliced genes in the human genome is much higher than previously thought.

Alternative splicing, RNA editing and other post-transcriptional modifications can result in product diversity that explains some of the complexity that Arctander is looking for. With the help of *C. elegans* he is now trying to understand the evolution of alternative splicing. One hypothesis is that splicing is a testing field, a source of relatively harmless variation, where selection can act slowly to increase the fractions of beneficial variants. But, surprisingly, this variation is hard to find. When comparing *C. elegans* with *C. briggsae*, two worms with an evolutionary distance similar to that of man and mouse, Arctander's group found alternative splicing to be astonishingly conservative. He says: "You should see the curves! Over all developmental stages all variants between the two worms are 95 % identically expressed!"

For Arctander, more information is hidden in the expression data delivered by microarrays than in the genomic sequence itself. However, to tackle the enormous amount of data, biologists are in desperate need of bioinformatics. So, in his view, the only way to extract information from this data is to integrate bioinformatics into daily work. "We have a close collaboration with the bioinformatics and PhD students who work in our group." But multidisciplinary work can sometimes be difficult: "I had a collaboration with mathematicians about small non-coding RNA identification and microarray analysis and in the beginning I was literally unable to communicate with them" Arctander says, laughing.

Besides alternative splicing, Arctander's group is studying regulatory RNAs. "We were looking for new regulatory RNAs for a long while, but didn't succeed. Fortunately others did, so we're working on microRNAs a lot now." Two questions keep microRNA researchers busy these days: How to measure expression of microRNAs using genome tiling arrays and how to find methods of detecting new targets of microRNA regulation. In 2006 Arctander's group made contributions in both fields (*Nucleic Acids Res.* 2006, 34, e107 and *BMC Bioinformatics*, 2006, 7, p.239).

The double-edged sword of specialising

Arctander says that "it can be unpleasant having to start from the beginning again, for example learning to grow worms and do microarrays. And it takes a lot of time – maybe 5-10 years – before you're so knowledgeable that your intuition works." Although the fundamental aspect of Arctander's work has always been understanding evolution by using molecular markers, his focus has kept changing constantly, as have his methods. Arctander: "Specialisation is a double-edged sword. You cannot test your hypothesis without experiments and you cannot experiment without specialising to some degree. On the other hand you have to avoid specialising too much and getting stuck in a very narrow field."

So Arctander believes that change is worth the trouble when it gives him the chance to get to the bottom of new questions. "All researchers should step aside once in a while and ask 'what do my results tell me about the broader perspective?'"

Brynja Adam-Radmanic