Int. J. Dev. Biol. 53: 725-731 (2009)
doi: 10.1387/ijdb.072575mr

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Molecular tools, classic questions -

an interview with Clifford Tabin

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ABSTRACT Clifford J. Tabin has made pioneering contributions to several fields in biology, including retroviruses, oncogenes, developmental biology and evolution. His father, a physicist who worked in the Manhattan project, kindled his interest in science. Cliff later chose to study biology and started his research career when the world of recombinant DNA was opening up. In Robert Weinberg's lab, he constructed the Moloney leukaemia virus (MLV-tk), the first recombinant retrovirus that could be used as a eukaryotic vector. He also discovered the amino acid changes leading to the activation of *Ras,* the first human oncogene discovered. As an independent researcher, he began in the field of urodele limb regeneration, and described the expression of retinoic acid receptor and *Hox* genes in the blastema. Moving to the chick model, his was one of the labs that simultaneously cloned the first vertebrate *hedgehog* cognates and showed that sonic hedgehog functions as a morphogen in certain developmental contexts, in particular as an organizing activity during limb development. Comparative studies by Ann Burke in his lab showed that differences in boundaries of *Hox* gene expression across vertebrate phylogeny correlated with differences in skeletal morphology. The Tabin lab also discovered a genetic pathway responsible for mediating left-right asymmetry in vertebrates; helped uncover the pathways leading to dorsoventral limb patterning; made contributions to our understanding of skeletal morphogenesis and identified developmental mechanisms that might underpin the diversification of the beak in Darwin's finches. Despite being a professor of genetics at Harvard, Tabin says: *"I have never done a genetics experiment in my life!"*. This is changing with his latest project: the genetics of Mexican cavefish. I interviewed Cliff on the 3rd October, 2007, in his office at Harvard.

KEY WORDS: *pattern formation, hox gene, evolution, development, positional information*

Could we start with some biography, and how you got into science?

I was born in 1954 in a suburb of Chicago, Illinois, called Glencoe. My father, Julius Tabin, was a physicist. He received his PhD at the University of Chicago at the dawn of the atomic age and then was a postdoc with Enrico Fermi[1] during World War 2 at Los Alamos. One of the things the Fermi group did on the Manhattan Project was measure the efficiency of the first test blast at Alamogordo which required getting samples from ground zero. My father was one of the people from the Fermi group who did this, going out in a lead-lined tank and taking a core sample only an hour after the blast. He received a half-lethal dose of radiation, which unfortunately meant that he was prohibited by the fledgling US Atomic Energy Commission from doing further experimental work with radiation for several years, ultimately leading to his leaving physics. Fortunately, however, he went on to live a long and rich life.

I grew up with my Dad teaching me about the world from a scientific perspective, and so I had a strong affinity with science from an early age. My earliest thoughts were either to become a scientist or a professional athlete. However, while I was a reasonable athlete, my talents were greater in the other arena. I went to the University of Chicago, following in my father's footsteps, and took a bachelors degree in physics with a double major in anthropology.

When it came time to apply to graduate school I thought about switching to biology. The interesting physics of the day was being done by large teams working on big machines, while biology

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Note 1: Enrico Fermi (1901-1954), an Italian physicist, recipient of the 1938 Nobel Prize for physics.

Published online: 9 June 2009.

ISSN: Online 1696-3547, Print 0214-6282

seemed to be a field where individuals could still design and carry out their own projects. In particular, biophysics seemed like an attractive opportunity, and hence I went to MIT [Massachusetts Institute of Technology], thinking I might work with crystallographer Alex Rich[2], the discoverer of Z-DNA. However the timing of my entry into graduate school led me in a different direction. This was 1976. Recombinant DNA had just been invented, and people were just starting to sense the power of this extraordinary new technology. The opportunities that afforded — to ask questions that were previously completely inaccessible — became vastly more appealing to me than crystallography had been.

Initially, you still had to do recombinant DNA research under P3 containment conditions in the lab — biological warfare was P4 — and you needed special licenses. There were only a couple of P3 facilities in the USA, and one of them was at the Cancer Center at MIT. I was attracted by the work being done by Bob Weinberg and David Baltimore on retroviruses. I did some experiments with each of them, most significantly modifying Moloney leukaemia virus so that it would transduce thymidine kinase, thereby creating the first retroviral vector at MIT (several other labs did similar work elsewhere). This was essentially just a proof of principle, making a vector to show it could be done. But then, Bob discovered human oncogenes (Shih and Weinberg 1982), which was a major breakthrough in the early research into the genetics of cancer. The core of my thesis became showing which amino acid changes led to the activation of the first known human oncogene, *Ras* (Tabin *et al*. 1982a).

As I neared the end of my graduate studies, I started to think about where I wanted to go with my research in the long run, now that I had mastered some of these extremely powerful recombinant DNA techniques. As I viewed it, the really big biological questions were *'where do babies come from?'* and *'where do species come from?'* Both embryogenesis and evolution seemed to be intimately tied to the regulation of morphogenesis. That became the issue I wanted to pursue. However, developmental biology of higher organisms was still largely unexplored. For example, this was before mouse knockouts, and gene transfer into developing chicken embryos. I joined Doug Melton's lab and I was there as a postdoc for just about a year.

The homeobox genes had only just been cloned. We had no idea about clustering or colinearity of the *Hox* genes in vertebrates and *in situ* hybridization hadn't yet been worked out in *Xenopus* because the eggs were so yolky. Doug's idea was to keep injecting homeobox genes into *Xenopus* eggs and see what phenotype you get. Richard Harvey and I in his lab set about cloning *Hox* genes and injecting them into eggs (Harvey *et al*. 1986). Ultimately, this approach led to important discoveries in Doug's lab, and elsewhere. However, I thought of asking the question from another angle. In flies, homeobox genes were known to be involved in segmentation. And in the vertebrates, the limbs and vertebrae were segmental in some respects. So, I wanted to see if *Hox* genes played a critical role in the development of these structures.

Of course, the attraction of the limb was that there was already a tradition of classical experimental biology in both limb development and regeneration. Doug Melton said that *Xenopus* was not suitable for limb regeneration, and suggested that, if that was what I wanted to pursue, I should find another lab and another system. The problem was, at that time, there were classical chick limb labs, but they were not doing anything on a molecular level; and there were molecular biology groups but they didn't know anything about morphogenesis. There really was no ideal lab for me to join.

So I applied for my own independent postdoctoral fellowship at Massachusetts General Hospital to try to do molecular limb regeneration studies in newts. Although I was initially unaware of it, at the same time another person, Jeremy Brockes, was also developing efforts to study limb regeneration at a molecular level. Once I heard of his work, I asked if I could go to his lab for a short stay to train in classical regeneration techniques. He was very gracious and very welcoming and I went over there and spent a month in London. When I got back to the USA, I cloned the first *Hox* gene differentially expressed in newt limb regeneration (Simon and Tabin 1993) (Jeremy's lab did similar work in the same time frame).

Then, Ron Evans and Pierre Chambon co-discovered the retinoic acid receptors (Giguere *et al*. 1987; Petkovich *et al*. 1987). Retinoids were known to have powerful effects when applied to regenerating limbs. I collaborated with Ron in cloning a retinoic acid receptor in regeneration (Giguère *et al*. 1989). I also showed that I could make transgenic newt blastema cells on the basis of pseudotyping. That means you infect the same cell with a retrovirus and a rhabdovirus and you get particles out that have a retrovirus genome in a rhabdovirus coat, and the latter has a much broader host range; so the mixed viruses carry a retroviral vector genome but can get into any cell. I got little blue newt cells, which looked amazing, but the efficiency was low and so I didn't publish the data. It did, however, demonstrate that I could genetically manipulate regenerating limbs, and hence helped get me a faculty job.

While finishing my post doc, I went to a limb meeting in Santander, Spain[3]. The American scientists working on *Hox* genes were immediately very excited by what I was doing. However some of the more classical American researchers in the limb field were, at first a bit stand-offish. Now, of course, I am good friends with many of them. However at the time the contrast was striking with many of the British limb people, who were very welcoming. Cheryll Tickle was extremely collegial and took the time to chat with me at great length. And Lewis Wolpert was just — Lewis! (for a recent interview with Lewis Wolpert, see Richardson, 2009). He criticised everything I was doing, in his somewhat condescending manner, but it was clearly done in the sense of an intellectual challenge and I enjoyed the banter with him. He was certainly accepting (treating me, for example, as he would any junior scientist who had been working for several years in a limb lab in Britain). And, as I said before, Jeremy Brockes was great.

Having started a molecular developmental system from scratch, I was in a relatively strong position when I started looking for faculty jobs and had some excellent opportunities. My best friend at graduate school was Connie Cepko and we long thought it

[2] Alexander Rich and colleagues reported the molecular structure of left-handed, double helical (Z-) DNA in 1979 (Wang *et al*. 1979).

[3] Developmental Patterning of the Vertebrate Limb: *NATO Advanced*. Research Workshop, Santander, Spain. September, 1990. See (Hinchliffe *et al*. 1993).

Fig. 1. Clifford J. Tabin *(c. 1999).*

would be fun to have adjoining labs some day. She was already at Harvard. When I got a job offer in the same Department my fate was pretty well sealed. I did however briefly waver between Harvard and two other possibilities. One was at Northwestern. While an excellent school, the particular Department interested in me was not all that strong. However it is in Evanston, Illinois, very near to where I grew up and would have meant going home, at some level a very appealing idea then (and now as well). I was also very flattered that MIT expressed an interest in me, since that was where I had done my graduate work.

As for MIT, they asked Jeremy Brockes for a recommendation. He gave a very balanced view of course, but said — quite rightly — that there was a mistake in our retinoic acid paper published in *Nature* (Giguère *et al*. 1989). It turned out that our 'newt retinoic acid receptor' was a composite of sequences from two different, related genes. We had cloned them as non-overlapping fragments that, together, gave a full retinoic acid receptor sequence. We didn't know it was a gene family and hence hadn't done a Southern blot with each half. If we had, we would have seen that they hybridized to different places in the genome. So I had to publish a little note in *Nature* — it wasn't a retraction — saying it was two different genes (Giguère *et al*. 2007), and that all the expression data in the paper related to only one of them. We now know them as RAR-β and RAR-γ. There was nothing wrong with the data we published, but MIT said the omission was sloppy and withdrew the offer of a faculty position. In truth, I was not as rigorous as I should have been, but I am not convinced it was something that should have changed their evaluation of my candidacy. This was not long after the Baltimore and Imanishi-Kari scandal[4] and they may have been still jumpy. In any case, I immediately shared the information about the *Nature* paper with Phil Leder, Chair of the Department at Harvard. Happily for me, he said that it did not affect his perception that I would be a good addition to his Department, and the offer from Harvard was still on the table. I had several other options as well, but did not consider any of them very seriously.

Sonic hedgehog and the chick limb

So I came to Harvard. Initially my plan was to continue with limb regeneration in the newt (Giguère, 1989). Chick limb development was the other classic limb field besides newt limb regeneration. Rather quickly, because of advantages in gene transfer, we switched to the chicken system. We started on this track because Connie Cepko, who indeed now had a lab next door, had developed mouse and chick retroviral vectors as tools for lineage analysis. These gave efficient gene transfer (which we had never achieved in the newt) but did not subsequently spread to neighbouring cells.

I thought we could do something similar, but with replication-competent viruses that could spread between cells. In principle I hoped we could use this approach to make transgenic limbs to test gene function. This indeed worked (Morgan *et al*. 1992), and opened up the chick system to the variety of developmental studies subsequently undertaken by a large number of labs. We also taught ourselves classical techniques such as grafting of beads in the chick limb, to complement our molecular skills. Because of our abilities to combine classical and molecular analyses, when we later came to discover *sonic hedgehog*, I took pride in the fact that nobody else in the world was better set up to take advantage of it.

I had a postdoc, Bob Riddle, who became interested in a *Drosophila* gene called *hedgehog*. We knew it was expressed in the posterior of the wing disc where it acted as a signal in organizing wing pattern. In this role, *hedgehog* seemed to be acting, at least superficially, in an analogous manner to an important activity in the chick limb. Classic experiments by John Saunders (For interview with J. Saunders, see Fallon, 2002) had shown that a region in the posterior of the chick limb bud, called the 'Zone of Polarizing Activity', or ZPA, was responsible for signalling differences between the digits in the developing limb bud (Saunders, 1977). Bob therefore decided to see if he could identify a homologue of the *Drosophila hedgehog* gene active in the posterior chick limb bud.

Our attempt to isolate vertebrate *hedgehog* homologues got an unexpected but critical boost in a round-about way, via some zebrafish work we were doing, so I have to backtrack a bit here. Investigators in Oregon had been screening for zebrafish mutations on a small scale for some time, and there were discussions

[4] That case involved allegations that data had been fabricated. These accusations were thrown out by an appeals board. See Friedly 1996; Kevles 1998; Turney 1998.

in Cambridge and Tübingen about scaling this up to the level of a large scale saturation screen. It was clear that a genetic map would be critical to eventually isolate the genes responsible for any interesting mutations that came out of these screens, but at this early stage, none of the central zebrafish labs were actively trying to make such a map. I thought that if we contributed a map to these efforts we might eventually be in a position to collaborate in looking at fin mutations (the equivalent of limb mutations) that emerged from the screens. So we pulled out microsatellites in zebrafish and showed that they had CA repeats and were polymorphic and that different strains have different alleles. We published a paper saying that you could use the same reagents to make genetic maps in fish that you did in humans (Goff *et al*. 1992).

We did not end up pursuing this further. However because of this pilot zebrafish mapping study, I was invited to a meeting that turned out to be of pivotal importance for the *hedgehog* project. Christiane Nüsslein-Volhard, who was the driving force behind the planned Tübingen screen, organized a meeting at Ringberg Castle in Germany (see Hafter and Nüsslein-Volhard, 1996). One evening, at dinner at that meeting, I happened to be sitting next to Andy McMahon and Phil Ingham. Phil had done important early work on the *Drosophila hedgehog* pathways, so I wanted to pick his brain a bit. He was gracious but he said that, although he was certainly happy to answer any of my questions, I should know that he was also attempting to clone vertebrate hedgehog genes. Then, Andy chimed in that he was as well!

For a moment, I had the sinking feeling that everyone at the entire table was secretly cloning *hedgehog* genes. Thankfully, it was just the three of us, and it turned out that we each had different biological questions and model species. Therefore, we were not competing with one another and decided to work together. We all clicked personally, and shared information very openly. The first to clone '*the*' vertebrate *hedgehog* was Andy (we later named his gene *desert hedgehog*). The only place it was expressed was in the testis, which didn't help very much with understanding limb development!

Fig. 2. Clifford J. Tabin.

BOX 1

CLIFFORD TABIN: SELECTED BIOGRAPHY

1954	Born Glencoe, Illinois
1976	Ph.D. student, MIT (under Robert Weinberg and David Baltimore)
1984	Postdoc In Doug Melton's lab, Harvard University
1985-88	Independent postdoctoral fellow, Massachusetts General Hospital
1989	Became faculty member at Harvard University
2007	Became Chair, Department of Genetics, Harvard University
2007	Became Member, National Academy of Science, USA

So, Bob designed PCR primers in my lab and got 3 different bands in the chick, suggesting that there were actually multiple *hedgehog* family members in vertebrates. Phil's lab quickly took advantage of this information to see where each was expressed in the zebrafish. Using *in situ* hybridization, he found one to be expressed in the notochord and floor plate. We pulled that same homologue from a limb bud cDNA library. We also knew that there was ZPA-like activity in the floor plate from published reports of grafting experiments, so it started to fall into place.

Bob and others in my lab showed that the hedgehog gene, which we dubbed 'Sonic hedgehog' [Ed. after the video game character "Sonic the Hedgehog" (Sega Corporation)], was indeed expressed specifically in the ZPA and was itself sufficient to organize the pattern of the digits of the limb. Parallel work focusing on the role of *sonic hedgehog* in the neural tube was carried out in Phil's and Andy's labs, leading three back-to-back *Cell* papers from our respective labs (Echelard *et al*. 1993; Krauss *et al*. 1993; Riddle *et al*. 1993).

Left-right asymmetry

The other big splash we had around at that time was the first molecular paper on left-right asymmetry. In the chick, we saw that *sonic hedgehog* is expressed on the left side of Hensen's Node — a small structure in the center of the early gastrulating embryo. Interestingly, it does not show this asymmetry in the mouse. Mike Levin joined the lab and he followed it up. I felt that, in order to make a story, we had to have a control gene that was expressed bilaterally and symmetrically at the node. Otherwise, if I published an asymmetric pattern people would say: 'you don't know how to do an *in situ*'. Ultimately, we obtained the type of control I wanted, *cNot1* from Mike Kessell. Before this, however, in an attempt to find a control, I called Claudio Stern — an expert on chick gastrulation. To my surprise, he said: 'I don't have a bilateral marker but I do have an asymmetric marker, an activin receptor. It is just sitting in my freezer. Do you want it?'

When we examined it, this receptor turned out to be on the right side whereas *sonic* was on the left. We were able to

BOX 2

CLIFFORD TABIN: SELECTED RESEARCH LANDMARKS

1982	Construction of first retrovirus (MLV-tk) that can function as a eukaryotic vector	(Tabin *et al.* 1982b)
	Analysis of the mechanism of *Ras* oncogene activation	(Tabin *et al.* 1982a)
1989	Cloning of Hox genes differentially expressed in regenerating newt limbs establishing molecular approaches could be used in the context of classic limb systems	(Tabin 1989)
1992	The first use of retroviral vectors to test gene function during development, opening up the chick to molecular analyses. Used recombinant retrovirus for ectopic expression of *hoxd11* in the chick wing	(Morgan *et al.* 1992)
1993	Cloning of *sonic hedgehog*, and functional evidence of its role in anteroposterior limb patterning. This was published back-to-back in *Cell* with articles, from the McMahon and Ingham labs, also reporting the cloning of vertebrate hedgehog cognates (Echelard *et al.* 1993; Krauss *et al.* 1993).	(Riddle *et al.* 1993)
1994	Discovery of a feedback loop (FGF-4 and SHH) between the apical ectodermal ridge and ZPA, two key signalling centres in the limb bud	(Laufer *et al.* 1994)
1995	The first identification of genes expressed left-right asymmetrically in the developing embryo and discovery of the pathway controlling left-right asymmetry in vertebrates	(Levin *et al.* 1995)
	Comparative molecular evidence for a spatial correlation between early *Hox* gene expression and adult skeletal morphology	(Burke *et al.* 1995)
	Identification of the genes establishing the dorsal-ventral axis of the limb bud	(Riddle *et al.* 1995)
1996	Finding that the gene *patched* is the receptor for Sonic hedgehog	(Marigo *et al.* 1996)
	Discovery that a second hedgehog family member, Indian hedgehog, regulates the rate of cartilage differentiation and the width of the growth plate in a feedback loop with a second signal, PTHrP	(Vortkamp *et al.* 1996)
1998	Identification of a transcription factor, Pitx2, specifying left-specific morphogenesis downstream of the left-right signalling pathway	(Logan *et al.* 1998)
1999	Discovery of a transcription factor, Pitx1, acting to make the hindlimb different from the forelimb	(Logan and Tabin 1999)
2001	Discovery that Wnt9a (formerly Wnt14) and β-catenin signalling is sufficient to direct prechondrogenic cells to form a joint	(Hartmann and Tabin 2001)
	First identification of a marker for tendon progenitors and analysis of tendon specification in the limb	(Schweitzer *et al.* 2001)
2002	Lineage analysis demonstrating that "myoblasts" that migrate from the somite into the limb bud do not have any patterning information and moreover are not yet specified to form muscle as opposed to endothelial tissue	(Kardon *et al.* 2002)
2003	Analysis of tendon specification in the trunk and discovery of a new compartment in the somites, the syndetome, of tendon progenitors	(Brent *et al.* 2003)
	Discovery of a cell population in the limb bud responsible for directing the pattern of the forming muscles	(Kardon *et al.* 2003)
2004	Analysis of the mechanism by which the SHH-Fgf feedback loop is terminated late in limb development, providing insight into the control of the length of the limb	(Scherz *et al.* 2004)
	Discovery that the ZPA cells undergo an enormous expansion within the limb bud resulting in the formation of a temporal gradient in addition to a spatial gradient of Sonic hedgehog in specifying digit pattern	(Harfe *et al.* 2004)
2004-06	Developmental mechanism underlying beak diversification in Darwin's finches	(Abzhanov *et al.* 2004; Abzhanov *et al.* 2006)
2005	Elucidation of the role of mir196 as a fail-safe mechanism for keeping forelimb-specific genes off in the hindlimb	(Hornstein *et al.* 2005)
2006	Development of the cave fish as a genetic system for studying evolution of morphological traits	(Protas *et al.* 2006)

These are some of the key discoveries that Tabin participated in, either as group leader, or first author. The current nomenclature for *Hox* genes (Scott 1993) is used below, and may differ from that given in the original paper. For the current wnt nomenclature, see http://www.stanford.edu/~rnusse/wntgenes/

connect these small expression asymmetries to one another in an epistatic pathway. But they were still quite small domains and their significance for broader left-right asymmetry was unclear. Meanwhile, still looking for a symmetric control, another postdoc in my lab, Randy Johnson drew my attention to a gene called *Nodal*. Based solely on its name, it seemed *that Nodal* should be expressed at the node. So, naively, I got a probe from Michael Kuehn to try as a symmetric control. Stunningly, however, when we examined its expression at different stages, we found that *Nodal* is expressed in an extraordinary, asymmetric pattern throughout the entire left side, and is completely missing from the right (Levin *et al.* 1995). We were able to use beads and antibodies to show that *Nodal* was downstream of *sonic hedgehog*, and formed a functional laterality pathway that ultimately controlled the handedness of heart looping.

Evo-Devo

What about your 'Only Five Fingers' paper on limb evolution (Tabin 1992)?

Denis Duboule still gives me hell about that! (for a recent interview with D. Duboule, see Richardson, 2009). I love talking to Denis, we often exchange long emails discussing ideas, raising issues contained in each other's papers etc. Before we knew what the *Hox* genes really did, Dollé and Duboule had demonstrated there were five *Hox* genes expressed in domains that formed a nested set in the early limb bud (Dollé *et al.* 1989). It struck me that this might provide a solution to an old puzzle: it is actually relatively common for people to be born with extra fingers. When they occur they are morphologically duplicates of existing digits, the most common being extra thumbs (preaxial polydactyly). It also turns out that it is apparently sometimes evolutionarily advantageous to have more than five digits. A six digit pattern has evolved at least twelve independent times in living mammalian groups; the most famous of these being the "thumb" of the panda. While the original five digits are kept for digging, the "thumb" is used to grasp Bamboo.

The thing is, in every one of these cases, the new digit is a modified wrist bone (a radial sesamoid in the case of the panda). This renders it less useful than a true finger or toe with joints. This begs the question of why evolution constructs such inferior appendages when adding another true digit (polydactyly) is genetically easy to achieve. The first half of my argument, which I still believe must be true, was that it must be easier to modify a wrist bone to a new function than to alter a polydactylous digit to a new purpose. The reason I gave for that being true was that there are only five *Hox* genes expressed in the limb bud and hence (I proposed) only five possible codes for finger types. Thus you can make a new finger but you can not make it distinct from the one next to it. Unfortunately, it

turns out to be wrong, because *Hox* genes don't work like that. They actually seem to have more to do with proximal-distal (shoulder-to-finger tips) differences, not differences between finger types.

At that time, a lot of chick people were not interested in evolution; they were focused on pattern formation.

Right. In a funny was this is almost cyclical. A generation before, when Ed Lewis did his pioneering work on *Hox* genes, so pivotal to understanding development, he was actually interested in an evolutionary question, arguing (quite correctly as it turned out) that the genes that specified different *Drosophila* segments were evolutionarily related through gene duplications. In any case, I had always been interested in evolution as well as development. I have always had in my lab, from the start, at least one card-carrying evolutionary biologist. The first was Ann Burke, who had done a postdoc with Bryan Hall. She came into my lab to do a comparative study of *Hox* gene expression (Burke *et al*. 1995).

I think that was a landmark paper, showing that *Hoxc6* shifted up and down the primary axis in accord with forelimb position. In fact, it is one of the earliest comparative studies that correlates expression patterns with resultant morphological differences between species.

Right, although one has to note parallel studies conducted around the same time by Michalis Averof and Nipam Patel comparing *Hox* genes in different arthropod groups (Averof and Patel, 1997). After Ann, another real Evo-Devo person in my lab was Arhat Abzhanov, who came from Tom Kauffman's lab in Indiana. His project in my lab was to try to uncover the developmental basis for the differences in beak shape in different species of Darwin's finches (Abzhanov *et al*. 2004, 2006). The idea to look at this question came out of a discussion I had with Marc Kirschner, who had just written an important synthetic book on evolution with John Gerhart (Gerhart and Kirschner, 1997). In discussing the evolutionary plasticity of the neural crest, they had an extended passage on Darwin's finches. Marc asked me: *'Do you know enough about development that you can think of what genes might be responsible for beak evolution?'*. I thought this would be a great thing to try. It was appealing in the same way that limbs had been, as a classic evolutionary context to bring molecular biology into.

Did you find subtle heterochronic shifts in the timing of *bmp4* expression between the finch species producing an adaptive response?

Yes, yielding different morphologies in the depth and thickness of the beak. Length has more to do with the calmodulin pathway, allowing the two morphological parameters to evolve independently of one another in response to environmental selection.

The most recent Evo-Devo problem we have been working on is a cavefish project (Protas *et al*. 2006, 2007). In this case we are trying to get at the genetic architecture underlying evolutionary change. When an organism invades a new ecological niche (an extreme case being entering a cave), some traits that were formerly adaptive will no longer be useful while other, new traits will be positively selected. Are the responses to changing selective pressures accomplished through a few genes or many? Are the genetic changes regulatory or structural? And if similar environments are invaded in different locations, are the same genetic pathways used to respond to them? The Mexican Cave Tetras (Protas, 2007) were a very interesting example to look at because the ancestral form is still around in adjacent rivers and are still inter-fertile with the cave forms. This has allowed us to use a genetic quantitative trait analysis to identify genes responsible for the evolutionary adaptations in this group.

The future

In terms of Evo-Devo studies, the tools are becoming so powerful: with RNAi you can make virtually any system into one that is open to molecular manipulation. And soon — perhaps in 5 years — we will be able to sequence any genome for an affordable price. While the push for this comes from medical applications, it will also mean that all species and indeed populations within species will be open to genetic and bioinformatics analysis. Even before this, with EST [expressed sequence tag] databases and annotated genomes of increasing numbers of model organisms, it is becoming increasingly easy to track down genes responsible for evolutionary transitions. At the same time, the methods for manipulating the genomes of the main model organisms have become so powerful that one can ask developmental questions with extraordinary precision. The principles we and others have uncovered, and the tools we have developed over the last two decades, have laid a groundwork. But, in my view, we are now entering a golden age for evolution and development studies.

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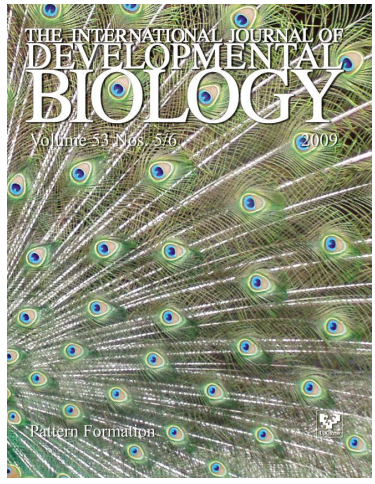

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