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The *Hox* Complex -

an interview with Denis Duboule

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ABSTRACT Denis Duboule is one of the most influential and highly-cited scientists in developmental biology. Born in Geneva in 1955, he holds dual Swiss and French nationality. His undergraduate studies in biology at the University of Geneva included research on mouse embryology. He later learned molecular techniques in the laboratory of Pierre Chambon, becoming a major player in characterising the newly-discovered vertebrate *Hox* genes. He helped discover their genomic clustering, realising that they had arisen by *trans* duplication. With Gaunt and Sharpe, he proposed that vertebrate *Hox* clusters might show spatial colinearity, and subsequently extended this concept to the timing of gene activation (temporal colinearity). Along with the Krumlauf laboratory, he reported the structural and functional conservation of the homeotic systems in flies and vertebrates. His lab was the first to describe nested patterns of *Hox* gene expression in the developing mouse limb, and later showed that digit-associated *Hoxd* gene expression was lacking in zebrafish paired fin development. His concept of phylotypic progression helps explain major evolutionary developmental phenomena in terms of *Hox* gene regulatory networks. His research helped reveal that the genital tubercle may, like the limb, be patterned by *Hox* genes. His lab developed targeted meiotic recombination (TAMERE), using it to make profound advances in our understanding of *Hox* gene regulation. Remote enhancers linked to digit patterning have been uncovered, together with a likely mechanism for colinearity. Denis lives in Geneva with his wife Brigitte Galliot, also a scientist, with their four children.

KEY WORDS: *Duboule D., Hox, Evo-Devo, Richardson M.K.*

You and your wife Brigitte[1] are both professional biologists in Geneva, a University town with a distinguished history in the biological sciences; I noticed a *Boulevard Carl-Vogt* when I was walking here.

Yes, Geneva is well-known for sciences. In the eighteenth century, Charles Bonnet was working here on regeneration, and of course Carl Vogt was a big impetus, both for Geneva University and for European science, already, via his support to Anton Dohrn to found the marine station at Naples (Buscaglia and Duboule 2002). Later, his successor Herman Fol founded the marine station at Villefranche (France).

Did you always know that you were going to be a biologist? I mean, did you have a career mapped out when you were young?

No, I never had a vocation, as such. If anything, I felt closer to Jimmy Page [famous influential guitarist and composer, one of the leaders of the rock group *Led Zeppelin*, Ed.] than becoming a scientist. I always did a lot of sports, and I thought about taking that up professionally — as a teacher, not as a champion. I was also a naturalist; I used to go out collecting things in the countryside, especially wild fungi. In fact I really became interested in botany. At one time I was planning to bring the fungi systematics into a computer. It was the time when the first PCs were just appearing.

Anyhow, I had also thought of becoming a veterinary surgeon — perhaps at a zoo, so that I could cure crocodile teeth and things like that. Unfortunately the only school of veterinary medicine in Swit-

Abbreviations used in this paper: EMBL, European Molecular Biology Laboratory; EMBO, European Molecular Biology Organization; ftz, fushi tarazu gene; STRING, sequential targeted recombination-induced genomic approach; TAMERE, targeted meiotic recombination.

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Note 1: Brigitte Galliot is a distinguished scientist in the field of *Hydra* developmental biology. See for example: (Galliot *et al.* 1989; Kaloulis *et al.* 2004; Miljkovic-Licina *et al.* 2007). She is also Associate Editor for Europe for the *Int. J. Dev. Biol.*

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Fig. 1 Denis Duboule (right) sharing a glass of Champagne in the garden, with Pierre Chambon (2009). *Photo courtesy of Denis Duboule.*

zerland was at Bern, and the teaching there was all in German, and I wasn't very good at that. Also, for personal reasons, I was keen on staying at the Geneva university.

At university, I kept up sports for a while but it became too difficult to combine with studies. When I had to choose a lab for the masters, another friend of mine had heard about a research project in the lab of the just-arrived star Karl Illmensee and said this would be really interesting for me. I went to see and was immediately charmed by the fellow, probably because we had the same blue jeans! So I stayed, which meant that my first real practical training — and publications — were in mouse embryology (Duboule *et al*. 1982a,b). But you know there was all that trouble about scientific fraud …

No, I don't know about that. Do you mind talking about it?

No, you can mention it. There was a scandal about scientific cheating in the Illmensee lab, and the problem is that I was one of the two…. what is the English word … *whistleblowers*, which turned out to be almost worse than being a faker. Karl Illmensee was, apparently, the first person ever to clone mice. He reported the clones in *Cell* in 1981 (Illmensee and Hoppe 1981), but the scandal blew up in 1982 (Budiansky 1983; Illmensee 1984; Marx 1983; Newmark 1985; Norman 1984), on a different issue, though. You can read a short account of this in *Clone* (Kolata 1997). Of course this made it impossible to find experts for my Ph.D. thesis because I was associated with this controversy and people were scared to take a position. Charles Babinet, from Pasteur eventually accepted and I could defend my PhD, thanks to him, and in the absence of my supervisor. Sadly, Charles died in April, 2008.

There was a commission that met in Geneva and London to investigate the fraud allegations, and one of the members was Pierre Chambon. I was getting quite depressed at that time by the whole thing and really thought of giving up, in particular as Illmensee still had strong support. Pierre realised this and came to my rescue by offering me a postdoctoral position in his lab [in Strasbourg, France]. I went there with an EMBO fellowship, after John Tooze had told me over the phone: *'for Christ's sake, take the money and go there!'*.

In the Chambon lab

Denis Duboule's association with *Hox* genes began in Strasbourg. This was an exciting time in developmental biology: the first cloning of *Drosophila* homeobox sequences was reported simultaneously in 1984 by the Scott lab in the USA and the Gehring lab in Switzerland (McGinnis *et al*. 1984b; Scott and Weiner 1984). Vertebrate cognates, including murine *Hox* genes, were also soon cloned in the Gehring and De Robertis labs (Carrasco *et al*. 1984; McGinnis *et al*. 1984a).

I notice your papers from this time are mostly not co-authored by Pierre but you do acknowledge him: were you functioning as an independent researcher?

Oh yes, Pierre wasn't primarily interested by *Hox* genes. At that time, he was just at the turning point in the discovery of nuclear receptors, after many important contributions to the field of transcription at large. He nevertheless felt the interest of this new field and anticipated the existence of large clusters in vertebrates, hence he advised me to screen a mouse cosmid library, made available by Hans Lehrach at the EMBL (Poustka *et al*. 1984). I was a mouse embryologist and I was aware of Ed Lewis's work, so this seemed like a great opportunity to learn molecular biology. I didn't have a clue about cosmids and genes and hybridisation but I just said "yes, I'll do that'!

So, we screened the library at low stringency with *Drosophila ftz, Antp and Ubx* probes from Walter Gehring and we started pulling out mouse cognates. And because we had large genomic fragments, up to 50 kb — a mouse *Hox* cluster, remember, is only of the order of around 100 kb — we could find contiguous genes on the same genomic fragments. So we could see that there was large clustering of these genes. At that time, the Dado Boncinelli, Frank Ruddle and Peter Gruss laboratories were also putting *Hox* clusters together, using phage libraries. I wrote a draft of the first paper on the work reporting a large cluster of *Hox* genes and I spent some hours sitting in Pierre's office, next to him. He corrected virtually every sentence and eventually told me: *'this is your work, I wont' sign the paper'*. He certainly did not need more papers but still, I thought this was *assez élégant*.

It is difficult to understand now how surprised people were by the finding of vertebrate cognates of *Drosophila* homeotic genes; it was quite amazing. Today we take the colinearity and clustering of vertebrate *Hox* genes for granted, and everyone thinks that it was a logical step after cloning *Drosophila* homeobox sequences in 1984. But it wasn't, mostly because our minds were not prepared for this. It took another 5 to 6 years to get a first general feeling, and even the book I edited in 1994 about homeobox genes (Duboule 1994a) came probably still too early, in terms of conceptual framework; it became rapidly obsolete.

At the end of my period in Strasbourg, we realized there was an *AbdB*-like gene in one of the mouse clusters, hence the two *Drosophila* homeotic clusters were one and the same in vertebrates. Sequence comparisons with similar data obtained by Dado Boncinelli in humans revealed that both systems were structurally very similar to each other.

Group leader in EMBL

Things were starting to come together in the vertebrate *Hox* field when the *Development* Supplement on Segmentation came out in 1988. We published there our work with Steve Gaunt and Paul Sharpe suggesting, for the first time, that spatial colinearity

was also at work in vertebrate *hox* genes (Gaunt *et al*. 1988). Soon after I moved to EMBL, we reported the functional similarities and evolutionary conservation between the systems in flies and vertebrates, along with Krumlauf lab (Duboule and Dolle 1989; Graham *et al*. 1989). I started working with Juan-Carlos Izpisua-Belmonte and Pascal Dollé — Pascal had followed me from Strasbourg, whereas Joska Zakany joined soon after.

These three collaborators really started the work on limb development, in particular Pascal who looked at *hoxd* gene expression in mouse limbs and did 3D reconstruction of the expression patterns. In this paper, we reported the temporal activation and showed that the same developmental genes could be recruited to do different things in a developing animal. These results were not really consistent with a simple AP gradient of positional values, as predicted by the positional information model; so we sent the results to *Nature* (Dolle *et al*. 1989) and I guess that the manuscript was circulated around Lewis Wolpert's *F1* generation of former colleagues because Julian Lewis asked me to come to Oxford to give a talk and explain our findings. I thought this was a nice way of doing things. After the talk (and a long discussion) Cheryll Tickle came up to me and said: *"Prof. Wolpert would like to meet you".*

I went to London to see Lewis. I walked into his office, and without looking up at me from his desk, he said: *"I don't believe a word of what you are saying! Please, sit down"*. He and Cheryll then kindly offered me a collaboration, leading to those papers where we showed that mirror-image duplications in the chick wing were correlated with mirror-imaging of the pattern of *Hox* gene expression (Izpisua-Belmonte *et al*. 1991). We also found that *Hox* genes were expressed in the genital tubercle and that this axial structure has morphogen activity when transplanted to the chick limb (Dolle *et al*. 1991b), which was explained many years after, once *sonic hedgehog* was found expressed there as well. I suppose my friend Lewis and I never agreed on the significance of any of these results, even late at the bar. But most importantly, this gave me the unique opportunity to access the remarkable community of British developmental biologists, where I made many other friends.

Fig. 2. Denis Duboule (left) with Ed Lewis (2001). *Photo courtesy of Denis Duboule.*

TAMERE and chromosome engineering at geneva

A major milestone in the Duboule lab was the development of the technique TAMERE for chromosome engineering in mice. This process has proved to be incredibly powerful for the analysis of the *Hox*D gene cluster, and has been the basis of many of Duboule's high profile papers of the last decade. It has helped uncover several regulatory interactions that influence transcription in the *HoxD* cluster, as well as revealing a likely mechanistic basis for colinearity.

How has TAMERE influenced the models of collinear gene regulation in both the trunk and the limbs?

The development of a targeted meiotic recombination system (Herault *et al*. 1998) was a key step, as it allowed us to plan and start a 15 years-long experimental strategy. If one wants to understand how neighbouring genes are co-ordinately regulated in time and space, it is crucial that one can change this neighbourhood, play around with genes, remove some or add others. This was a long commitment and many gifted and brave collaborators participated. Recently, our new STRING approach [Ed. sequential targeted recombination-induced genomic approach] (Spitz *et al*. 2005) has complemented TAMERE. The allelic series is now almost fully produced (we keep about 150 *HoxD*-related lines in the laboratory) and we progressively enter into an analytical phase, which will likely last for another 10 years. Yet we hope that the various collinear mechanisms will be cracked before this deadline, as was recently achieved for colinearity in developing digits, which could be modelled thanks to these numerous stocks of mice (Kmita *et al*. 2002; Montavon *et al*. 2008).

Evo Devo

What do you think of the current craze for Evo Devo? I know we have talked about this before, and we both agreed that Evo Devo papers are sometimes long on speculation and short on data.

I believe that a full understanding of fundamental mechanisms of pattern formation in one model organism would likely explain related mechanisms in other species. The epistemological question is whether or not one can fully understand one given mechanism without considering its realm of variations, i.e. without looking at many other species. This is the paradox of Evo-Devo, which I am convinced is merely a discipline of transition, which will soon be replaced by Evo-Genomics or something like this. But you are right in saying that the quality of the hypotheses, in this field, are often well above the significance of the data.

But note that the exact opposite can sometimes be seen with high throughput developmental genomics (whatever that means). In a congress I attended recently, huge amounts of robust datasets were presented. Yet these mountains of data were not placed in a conceptual landscape, to make them intelligible. I am personally more interested in understanding one thing in some depth in one system, then using this to build or adapt a conceptual

framework and then look around to see if it bears any heuristic value.

You did a very influential comparative study of zebrafish paired fin patterning — and the absence of tetrapod-like digit domains of *Hox* expression in the fin. Did you consider carrying on with the zebrafish model?

That was work where we suggested that a new, late phase of *hox* gene expression was associated with digit evolution in tetrapods (Sordino *et al*. 1995). I did consider continuing with zebrafish and in fact we set up a colony. Eventually, I saw how tiny the fin buds were and I changed my mind, even though they look nice and smell better than mice.

Scientific philosophy (the meaning of life)

When you were growing up, you had an interest in natural history and fungus taxonomy. But now as a researcher, you are strongly focused on the model systems approach rather than a comparative approach.

I guess you need to be a little bit obsessive in science! I tend to admire people who study one subject in great depth, for a long period of time, like an architect building a single cathedral in his lifetime, or Marcel Proust writing an almost unique *essai*, not even terminated at his death. I think that is an admirable thing to do — even if the cathedral is never built, eventually. This is a matter of intellectual adventure, a trip into ourselves, a quest for something we do not necessarily want to find. Unfortunately, the actual research structures make these trips understandably difficult. Nowadays, the *h*-index of Ed Lewis [Hirsch index, a measure of the apparent productivity and impact of a scientist, Ed.] would have caused him troubles!

Fig. 3. Denis Duboule in 2006 at the Ecole Polytechnique Fédérale de Lausanne, Life Science Faculty. *Copyright Alain Herzog.*

BOX 1

DENIS DUBOULE: BIOGRAPHICAL TIMELINE

1955	Born in Geneva (Switzerland)
1984	Doctorate of Science, University of Geneva Postdoctoral fellow in Strasbourg, Medical Faculty
1988	Group leader, EMBL, Heidelberg (Germany)
1992	Full Professor of Biology, University of Geneva
2006	Full Professor of Developmental Genomics, Federal Technology Institute, Lausanne (Switzerland); continues also as full Professor of Biology at Geneva University

The selection of a model system because it is the most 'suitable', because it has 'advantages' as we often hear here and there, is to me nonsense. There is no such thing as *advantages* or *disadvantages* in animals. There are adaptive traits, which facilitate the understanding of particular aspects such as very few cells in *C. elegans*; or a very fast, mother-driven development in flies. But if you like to get to the core principles, the general laws, you may as well select the most *unsuitable* model to make sure you will not spend too much time working out highly adaptive traits, not necessarily relevant to the central mechanisms. Yet these are theoretical considerations, of course, and most of the time, one does those things that can be done. Also one shouldn't be asked to justify the use of an experimental model. It is a great privilege to work with the model you simply like. I like mice because they are warm, sweet, cute and they watch me. In addition, they breed well.

I can see the power of model systems, but surely, for evolutionary biology, don't we also need comparative studies? I mean, look at Cliff Tabin with his chick limbs, Darwin's finches, and cavefish. Or Darwin, who studied everything from worms to orchids, and derived general principles by analysing all that variation.

I am not being judgmental about it, I am just telling you my own taste. Cliff has been very successful — and Darwin also did quite well. It is essential that all kinds of approaches and scientists address the same fundamental questions from different perspectives, as only this diversity will take us to the solutions. There is no unique truth in this process, we all are right in what we do, provided we do it reasonably well.

Why do you think *Hox* genes became so intensively studied? Because they are somehow unique and special?

I wish they would, but I guess it is much simpler: flies carrying mutations in these genes have gross morphological phenotypes, and so the mutant flies were easier to catch. This was already a good start. Now whether or not this explains the extraordinary epistemic value of this gene family, I really don't know. After the initial boom, *Hox* genes were not so intensively studied, after all. They came back on stage quite recently, along with modern technologies such as ChIP on chips [chromatin immunoprecipitation with microarray technology, used to investigate protein-DNA interactions *in vivo*. Ed.]. A range of key concepts was derived

BOX 2

DENIS DUBOULE: SELECTED RESEARCH LANDMARKS

1986	Evidence that vertebrate *Hox* genes have a role in pattern formation; cloning of mouse *HoxA* cluster	(Duboule *et al.* 1986; Gaunt *et al.* 1986)
1987	Mouse *Hoxa1* cloned and characterised	(Baron *et al.* 1987)
1988	First proposal of spatial colinearity in vertebrate *Hox* gene expression; Mouse *Hoxd4* cloned	(Gaunt *et al.* 1988; Featherstone *et al.* 1988)
1989	Cloning of murine *HoxD* cluster. Proposal of functional conservation between vertebrate and Drosophila homeotic systems (see also (Graham *et al.* 1989)); evidence that 5' *Hox* genes not closely related to *Antp* sequence; temporal and spatial colinearity seen in *Hox* gene expression in mouse limb	(Dolle *et al.* 1989; Dolle and Duboule 1989; Duboule and Dolle 1989; Galliot *et al.* 1989; Gaunt *et al.* 1989)
1989	Identification of *Hox4.5* suggests a *Drosophila*-like organisation	
1991	Identification of 5' murine *Hoxd* genes related to AbdB; temporal and spatial colinearity found in *Hoxd* gene expression along the trunk.	(Dolle *et al.* 1991a)
1991	Murine *Hox*d13 cloned, thereby identifying the 5' end of the *HoxD* cluster; similarities between the development of the limb and the genital buds	(Dolle *et al.* 1991a)
1993	Mutation of *Hoxd13* and heterochronic phenotype in limbs	(Dolle *et al.* 1993)
1992-1994 1994	The phylotypic progression concept (the hourglass) provides a unifying concept for many key phenomena in evo devo; concept of posterior prevalence proposed	(Duboule 1994b; Duboule and Morata 1994)
1995	Cloning and studies of zebrafish *Hox* genes; Digits as neomorphic structures; *laxitas terminalis*	(Sordino *et al.* 1995; Sordino *et al.* 1996)
1996	First chromosome engineering on *HoxD*	(van der Hoeven *et al.* 1996; Zakany and Duboule 1996)
1998	TAMERE (targeted meiotic recombination) developed; transitionism and constraints in development and evolution	(Duboule and Wilkins 1998; Herault *et al.* 1998)
2001	Link between *Hox* gene and the segmentation clock	(Zakany *et al.* 2001)
2002	Mechanism for colinearity proposed in terms of differential tropism of the digit enhancer for 5' *hoxd* genes	(Kmita *et al.* 2002)
2003	Digit enhancer found and characterized to be part of a larger global control region. Concept of regulatory landscape.	(Spitz *et al.* 2003)
2004	*Hox* genes organize limb AP polarity upstream *Shh*	(Zakany *et al.* 2004)
2005	*Hox*-less mice have virtually no limbs (double cluster deletion)	(Kmita *et al.* 2005)
2005	Use of STRING to split *HoxD* into two pieces	(Spitz *et al.* 2005)
2006	Characterisation of 'early phase' *Hox*d gene transcriptional activation in the limb and its function in patterning the distal stylopod and zeugopod described	(Tarchini and Duboule 2006)
2006	Molecular constraints during limb development and evolution	(Tarchini *et al.* 2006)
2007	A revised view of the structural and functional evolution of *Hox* clusters	(Duboule 2007)
2008	Mathematical model of colinearity; the origin of thumbness	(Montavon *et al.* 2008)

These are some of the key discoveries that Duboule participated in, either as group leader, first author or collaborator. The current nomenclature for *Hox* genes (Scott 1993) is used, and may differ from that given in the original paper.

from this gene family and I anticipate this will continue.

I notice that you sometimes put jokes[1] in your papers and their titles — Do you have a mischievous streak?

I was a fan of *Monty Python* even before I could fully understand their English. When I became a French *Chevalier*, a couple of years ago, my sons followed me for a while, doing clip-clop noises, as in *The Holy Grail*. Science is arguably a serious matter, but why should the *presentation* of science be so boring? I confess I went a bit too far with the subheading: *Anus horribilis* in one paper, which was about the abnormal and ugly development of the anal sphincter, published in a respectable English journal (Kondo *et al*. 1996) the same year when Windsor Castle caught fire. This reference to Queen Elizabeth's *Annus Horribilis* was moderately appreciated by some friends over there. I had assumed the Queen wouldn't read my papers.

[Note: At this point, we discussed some of the other jokes that are embedded in several of Duboule's articles. I hasten to point out that these do not in any way affect the science or arguments made in those papers. One such joke involves the TAMERE technique; another is a fictitious bibliographic reference containing a pun. We both agreed to leave the reader to discover these — and others — for themselves, as well as the *nom de plume* that Denis uses from time to time].

Are you optimistic about the future, and what do you see as the next wave in developmental biology and the study of pattern formation?

I am very optimistic about the future of basic research in these fields. There is still so much to discover. I am a firm believer in the mix between tradition and innovation. We should of course remember how nice it was in the past, but surely turn our energy to what comes next, because this is what counts: the interface between the now 'classical' developmental and molecular genetics and genome-wide approaches sounds terrific to me; working with genomes as we used to work with genes. I also see a lot of fun in the direct visualisation of developmental processes, at the cellular level, with new technologies, modelling, watching single molecules travelling from here to there.

On the other hand, I am generally quite pessimistic regarding what is happening with all kinds of religious, political or economic extremisms and I hope that free and happy scientists will still exist by the end of this century. If not, this would be too bad because what fun it is!

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[1] Some particularly memorable ones are: *No milk today, my Hox have gone away* (Duboule 1999); *Of fingers, toes and penises* (Kondo *et al*. 1997); *… the case of the caecum* (Zacchetti *et al*. 2007).

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