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A scientific journey in the garden of the *Hox* genes: an interview with Jacqueline Deschamps

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ABSTRACT For this Special Issue of *The International Journal of Develomental Biology* on *Hox* genes, the guest editors met Jacqueline Deschamps for an interview about her research career dedicated to understanding how *Hox* gene expression is initiated, maintained and functionally utilized in the mouse embryo. We describe here her journey through some of the main discoveries which led to our current knowledge about how *Hox* genes contribute to shaping the animal body plan. This journey was a human adventure also, of more than 30 years, in the light of which Jacqueline Deschamps delivers here messages to the younger generations of scientists.

KEY WORDS: *Hox, Cdx, Wnt, mouse embryo, body plan, embryo patterning, embryo elongation, gene regulation*

Jacqueline Deschamps is an important figure in the HOX world. Since 1985 she dedicated all her efforts to unravelling the fascinating questions about the initiation and maintenance of *Hox* gene expression in the mouse embryo, and the function of these genes during embryogenesis. She contributed to masterpieces in the field, showing the importance of inductive events at the onset of *Hox* gene expression (Forlani *et al.*, 2003, Neijts *et al.*, 2016), the role of CDX proteins (referred to "para-HOX" proteins) in the process (Neijts *et al.*, 2017), and the importance of *Hox* gene expression in elongating the embryo as well as in terminating its elongation (Young *et al.*, 2009). Jacqueline Deschamps's work has been seminal and inspiring for many in the HOX research field.

Jacqueline's wish has always been to address questions in the context of the embryo. While she considers that *in vitro* studies are important and can be of high heuristic value, she aimed at verifying data *in vivo* in the murine embryo whenever possible. This *credo* is the guideline she imposed on herself and which successfully allowed her to write some of the most beautiful pages of the HOX history.

We met Jacqueline on a sunny spring day in Belgium, her homeland, while on the way from the South of France to the Netherlands where she established her lab more than 30 years ago. A delightful interview with a great, generous, wise and sensible researcher (Fig. 1).

Jacqueline, when did you know you were going to become a biologist and a researcher? Was this an early wish rooted in your early years?

JD- Well, not really. I was actually interested in biology during my high-school years, but I first needed to convince my parents that I could become something else than a primary school teacher. I then went to study science at the university (Université Libre de Bruxelles-ULB- Belgium). At that time, I was advised not to engage in a Biology course, but rather to study chemistry, which would ultimately allow me to turn to biological chemistry and molecular biology. In Brussels, I was fortunate enough to have quite inspiring professors: René Thomas, a leading scientist in DNA biochemistry and bacterial genetics in Belgium; the Nobel laureate in Chemistry and Quantum Mechanics, Ilya Prigogine; and the renowned biochemist and embryologist Jean Brachet. However, the most influential professor for me personally has been the microbiologist and yeast geneticist Jean-Marie Wiame who succeeded in contaminating me with his fascination for microbes and their capacity to adapt to their environment. For this reason, I started research training under the supervision of Prof. Wiame, for a Master thesis first, and then for a PhD. Discovering

Abbreviations used in this paper: KO, knockout; NMP, neuro-mesodermal precursor.

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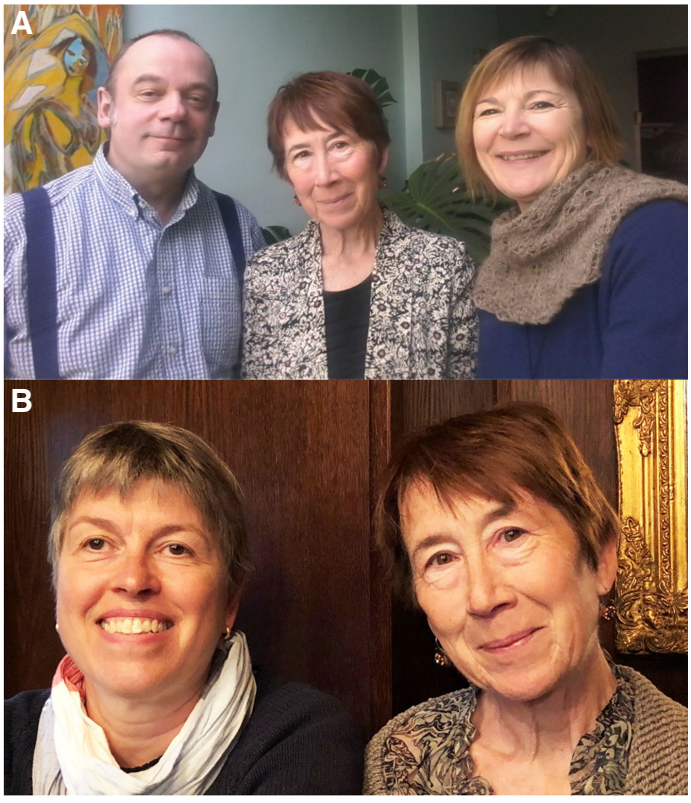

Fig. 1. Jacqueline Deschamps and the *Int. J. Dev. Biol.* Guest Editors for the Special Issue *"Hox genes: past, present and future of master regulatory genes".* (A) *From left to right: René Rezsohazy, Jacqueline Deschamps and Françoise Gofflot.* **(B)** *Lucie Jeannotte (left) and Jacqueline Deschamps (right). Belgium, 2018.*

the excitement of research was really a revelation. My research subject was on the genetic regulation of arginine catabolism in the yeast *Saccharomyces cerevisiae*. This involved mutant analyses, growth tests, dominance-recessivity tests and so on… nothing to do with molecular biology yet…neither with *Hox* genes. *Hox* genes entered my life some time later.

After my PhD thesis, I joined the laboratory of Arsène Burny to study the molecular relationship between the Bovine Leukemia Virus and its target host cells. This postdoc dipped me into molecular biology. The perspectives to stay and to obtain a position were weak at that time in Belgium without having a postdoc experience abroad. After several applications resulting in different potential opportunities, I chose to cross the ocean and move to the USA to land in Southern California, at the Salk Institute in San Diego. I joined Inder Verma's group to work on the transcriptional regulation of the proto-oncogene *c-Fos*. There, I identified an enhancer of *c-Fos* (Deschamps *et al.*, 1985), and this work really marked my commitment to the field of transcription. Another facet of my postdoc project was to characterize the expression pattern of *c-Fos* in the embryo by *in situ* hybridization. At that time, the embryo was basically unknown to me. I just remembered the brilliant lectures of Jean Brachet and his team at the university, but that was it. In the library's records of the Institute, I discovered the complete collection of the *Journal of Embryology and Experimental Morphology* (which has been continued as *Development* since 1986). This immediately stimulated in me a real passion and fascination for the developing embryo, the way it is organized step-by-step, the way tissues and organs are formed etc. This study on *c-Fos* was a trigger for the remaining of my research years: combining molecular biology and embryology, to understand the molecular clues underlying gene regulation and function during embryonic development.

When I envisaged returning to Europe to pursue my scientific career, I got the opportunity to work at the Hubrecht Laboratory in Utrecht, the Institute of the Royal Dutch Academy of Arts and Sciences dedicated to Developmental Biology. This was exactly what I could dream of, as it allowed me to study the mouse embryo at the molecular level of gene regulation. The Hubrecht Laboratory, now called the Hubrecht Institute, had a long and famous history in classical embryology. Pieter Nieuwkoop performed his pioneering embryology work there and he had been the head of the Hubrecht Laboratory from 1953 to 1980. In late 1985, the positions that opened at the Hubrecht were aimed at boosting molecular biology. This is how I got the chance to start my own group.

How did you get started with studying the *Hox* genes?

The years 1984-1985 were extremely flourishing for the nascent field of HOX biology. Only a few years after the discovery of homeotic genes (Lewis, 1978), the homeobox was discovered, almost at the same time by Gehring and colleagues (McGinnis *et al.*, 1984) and by Scott and Weiner (Scott *et al.*, 1983). In 1985, six papers appeared back to back in *Cell* showing the presence of *Homeobox* genes in the animal kingdom (Carroll and Scott, 1985, Colberg-Poley *et al.*, 1985, Hart *et al.*, 1985, Hauser *et al.*, 1985, Joyner *et al.*, 1985, Manley and Levine, 1985). It is in this context that I arrived at the Hubrecht (Lab). I had brought along from the US libraries of phage clones with genomic inserts from the mouse, with the aim of attempting to clone new *Homeobox* genes. That was the starting point of my research in the HOX field in late 1985.

The very first experiments we did with our newly cloned *Hox* genes were on *in vitro* models of the embryo, which were used at the Hubrecht, namely embryonic carcinoma cells and the newly discovered embryonic stem cells. We found that it was not cellular differentiation *per se* that was accompanied by a strong *Hox* gene expression, but addition of strong inducers like retinoic acid (Deschamps *et al.*, 1987). This was a first indication that *Hox* genes respond to inductive events, an indication that was confirmed later in the embryo. Looking at what happens in the embryo was actually my main interest. The hypothesis of a crucial role of the *Hox* genes in the early mouse embryo was already being investigated by Denis Duboule, together with Stephen Gaunt. They showed that *Hox* genes are expressed in the primitive streak during gastrulation (Gaunt *et al.*, 1986). With our *Hox* probes, and those we received from Brigid Hogan and Eddy de Robertis, among others, we carried on *in situ* hybridization on sections of early mouse embryos. A striking observation we made was that *Hox* gene expression did not simply follow cell lineages and cell migration, processes that had been well studied by our colleague expert in early mouse embryogenesis, Kirstie Lawson (Fig. 2). There were events in the posterior part of the primitive streak that seemed to elicit the emergence of *Hox* gene transcription (Deschamps and Wijgerde, 1993). This conclusion launched the grounds for questions I have followed until now: how are the *Hox* expression patterns initiated, established and maintained in the mouse embryo? How are these genes regulated in time during embryogenesis? And how are the spatial, 'Russian doll-like' *Hox* expression profiles generated?

Fig. 2. Jacqueline Deschamps (1997), *together with Kirstie Lawson (left) and Margarita Silió (middle) during a farewell party in Utrecht.*

In the late eighties, it became clear that the mammalian *Hox* genes are organized in clusters, similarly to what had been discovered in *Drosophila* (Duboule and Dollé, 1989, Graham *et al.*, 1989), and that their expression domains are collinear along the antero-posterior axis of the embryo (Gaunt *et al.,* 1988, Izpisua-Belmonte *et al.*, 1991) and some of the secondary axes such as the limbs (Dollé *et al.*, 1989). These were impressive series of discoveries, keeping the excitement vivid about the *Hox* genes in the developmental biology community (Fig. 3). I have been interacting with Denis Duboule and Robb Krumlauf ever since the late 80's. Robb hosted one of my PhD students for a while early on, and Denis welcomed one of my students for a short stay in his lab at some point. Denis' approaches and way of thinking have always been very much inspiring to me.

In the early 90's, I set up the technique of mouse transgenesis at the Hubrecht to perform studies on the transcriptional regulation of the *Hox* genes in the embryo (Vogels *et al.*, 1993), and to engineer gain of function mice as an approach to study the function of these genes (Charité *et al.*, 1994). This approach was soon complemented by the use of the gene knockout (KO) procedure to generate loss of function strains of mice. The first *Hox* KO experiment in my lab was the inactivation of *Hoxb8*, that allowed us to collaborate with Philippe Brûlet and Pierre Chambon who had inactivated *Hoxc8* and *Hoxd8*, respectively, and to study the results of the invalidation of all *Hox* genes belonging to the group 8 (van den Akker *et al.*, 2001). A number of other gene inactivation experiments later on concerned the ParaHox genes *Cdx* (van Nes *et al.*, 2006). Key functions of these genes were deciphered during the elongation of the embryonic axis (van Rooijen *et al.*, 2012), and during the specification of intestinal endoderm (Stringer *et al.*, 2012). The absence of *Cdx2* was shown to lead to the transformation of intestinal stem cells into gastric stem cells (Simmini *et al.*, 2014), in collaboration with Hans Clevers and his team at the Hubrecht.

Jacqueline, would you tell us what you think might be your main achievements in the HOX research field

I don't think it is up to me to answer this question! But I can tell you what excited me most for over 30 years of research. A first excitement was identifying signals which are the first inducers of *Hox* genes in the early embryo. As mentioned above, once we saw that *Hox* gene expression is not simply inherited through cell divisions and migration (Deschamps and Wijgerde, 1993), we hypothesized that inducers should act in a non-cell autonomous way in the posterior part of the primitive streak. This was confirmed by the work with Sylvie Forlani and with Kirstie Lawson (Forlani *et al.*, 2003), and we suspected that Wnt signaling could be a crucial actor in that respect. Ultimately we produced the *in vivo* conclusive evidence for the role of WNT3 in initial *Hox* gene activation (Neijts *et al.*, 2016). These latter studies, which involved sensitive technologies to characterize transcription, chromatin states and genome structure made use of a cellular model system derived

Fig. 3. **Beauty in Embryology, Patterning and Shaping the Body meeting** *at the Center for Developmental Biology, RIKEN, Kobe, Japan (2004) organized by Yoshiko Takahashi. Speakers at the meeting included (from left to right): (back row) Shinji Takada, Koji Akasaka, Shigeru Kondo, Hiroyuki Takeda, Yasumasa Bessho, Kohei Hatta, Shigeru Kuratani; (middle row) Masatoshi Takeichi, Andrew Lumsden, Jim Weston, Chuck Kimmel, Yumiko Saga; (front row) Yoshiko Takahashi, Nicole Le Douarin, Denis Duboule and Jacqueline Deschamps. Photo courtesy of Yoshiko Takahashi.*

from the embryonic epiblast, the Epiblast Stem Cells. The findings were then confirmed in the embryo *in vivo* by reporter assays and by CRISPR-generated mutants (Neijts *et al.*, 2016).

Complementary to this, another exciting issue concerned the involvement of CDX in controlling *Hox* gene transcription and function. The initial intuition we had was that candidate binding sites for CDX in the vicinity of *Hox* genes were relevant to their transcriptional regulation. With Jeroen Charité, we had shown that compromising or multimerizing these CDX binding sites modified both the timing and expression pattern of *Hox* genes in the embryo (Charité *et al.*, 1998). We later showed that CDX proteins actually bind at these sites in the vicinity of *Hox* genes *in vivo* (Amin *et al.*, 2016), and that these interactions mediate the induction of the *Hox* genes in a trunk segment (Neijts *et al.*, 2017), after their transcription has been initiated by Wnt stimulation earlier in the gastrulating embryo.

One more excitement came with the discovery that CDX and HOX gene products are essential for the generation of all post-occipital tissues, in addition to their patterning function (Chawengsaksophak *et al.*, 2004, van Rooijen *et al.*, 2012; both papers in collaboration with Felix Beck - Leicester). This role of the *Cdx* family in elongating the embryonic axis became even more fascinating when it appeared to be evolutionary conserved in bilaterians progressively extending their body from anterior to posterior.

Another spectacular outcome of these experiments was that upon causing the last *Hox* gene of any of the *Hox* clusters to be highly expressed at an earlier time point than they normally are, axial growth of the embryo stopped prematurely. To obtain precocious expression of posterior *Hox* genes at the start of these experiments in 2005, we thought of using in collaboration with Jean-Noel Freund (Strasbourg) the promoter of *Cdx2*, which we knew was initially expressed similarly to an early *Hox* gene. We found that advancing expression of *Hoxa13* by expressing it under the control of the *Cdx2* promoter led to a very severe posterior axial truncation of the embryos, and we obtained similar results with *Hoxb13*. It appeared that Moises Mallo (Lisbon) also had observed a severe posterior truncation phenotype upon overexpressing *Hoxc13* in embryos using another early promoter. This was the basis for a fruitful collaboration between my laboratory in Utrecht, and those in Leicester, Strasbourg and Lisbon (Young *et al.*, 2009). This dominant negative function of *Hox13* confirmed the importance of keeping the last *Hox* gene from the clusters silent until the embryonic trunk has been generated, via a mechanism called 'Posterior prevalence' that is also at work in *Drosophila*, and had already been described by Denis Duboule in different growing embryonic structures (Duboule, 1991, Duboule and Morata, 1994). The working mechanism of this antagonistic action of *Hox13* versus the trunk *Hox* genes and *Cdx* during axial extension has recently been found to occur at the level of the HOX13/CDX proteins (Amin *et al.*, 2016).

The remarkable discovery of the neuro-mesodermal precursors (NMP), or axial stem cells by Val Wilson (Cambray and Wilson, 2002, Cambray and Wilson, 2007) gave a new impulse to our *Hox/Cdx/Wnt* studies. These NMPs are the progenitors of mesoderm and neurectoderm of the embryonic trunk, and they localize to the primitive streak, the site of early *Hox* gene expression. In collaboration with Val, we then attempted to investigate whether ablation of *Cdx* genes, which arrests axial elongation, was at work via inactivating the NMPs. The answer was that it was not the case. The results confirmed that the process of *Cdx/Hox* -mediated tissue elonga-

Fig. 4. Jacqueline Deschamps's former team members. (A) *Those who did their PhD work in Jacqueline's lab came together on the occasion of her farewell symposium at the Hubrecht Institute in June 2016. Back row from left to right: Fried Zwartkruis, Ronald Vogels, Jeroen Charité, Johan van Nes, Anthony Oosterveen, Eric van den Akker. First row, right from Jacqueline: Monika Bialecka, Cesca van de Ven, Salvatore Simmini, and Roel Neijts. Not in the photo: Teddy Young. Courtesy of Jacqueline Deschamps.* **(B)** *Postdoctoral researchers and several of the technicians who worked in Jacqueline's lab. Front row: Carla Kroon, Antje Brouwer, Isabelle Valarché, Nathalie van der Lugt, Laura Zeinstra, (Jacqueline Deschamps), Sylvie Forlani. Back row: Wim de Graaff, Fried Zwartkruis, Jeroen Charité, Bernard Roelen, Mark Reijnen, Eric van den Akker and Anthony Oosterveen. Not in the photo: Shilu Amin, Carina van Rooijen, Jeroen Roelfsema. Photograph taken after the farewell symposium, courtesy of Jacqueline Deschamps.*

tion depends on signaling downstream of CDX/HOX (Bialecka *et al.*, 2010). *Cdx* mutations affect the niche of the axial stem cells.

Subsequently, integrating our data on early *Hox* gene expression and the localization and behavior of axial stem cells in the embryo, we proposed that 3' to 5' *Hox* genes are expressed in a temporal progression in the NMP region of the streak, 'instructing' the early to late axial progenitors. These instructions would be relayed by the descendants of these NMPs in the emerging axial tissues, generating the set of spatially collinear trunk expression patterns that we know (Deschamps and Duboule, 2017). To use a HOX 'jargon': temporally collinear *Hox* gene expression would be transmitted at the level of the axial stem cell NMPs into spatially collinear expression domains in embryonic tissues (Deschamps and Duboule, 2017).

In your opinion, what's next in the HOX field; what are the main questions remaining to be addressed in the context of HOX biology?

One of the great challenges will be to understand how all the *cis*-regulatory modules which have been and remain to be identified act in concert to provide cues for the developmental control of genes such as the *Hox* genes. Questions are thus how these modules cross-talk and physically interact in topological domains, how these domains change in time, and how the modules are "recycled" in late, post-embryonic, activities of the *Hox* genes which appear to be more important than initially expected. I thus believe that elucidating the complexity of *Hox* gene regulation still remains a challenge for the future.

Another important aspect of the biological function and working mechanisms of *Hox* genes has remained underexposed: the mode of action of HOX proteins, the actors in target gene transcription. One reason has long been the difficulty to generate specific anti-HOX antibodies. Recently, reports have been published that document how HOX proteins interact with partner proteins to exert their function (Amin *et al.*, 2015) and more studies should follow, which will help understanding the action of the *Hox* genes in all biological contexts where they are involved.

An important challenge today in biology in general is to make sense of the huge amount of data that becomes available through high-throughput technological approaches. So far, these data are only exploited superficially, and the next task will be to deepen the analyses. This is particularly important for the biomedical perspectives of the research, from the etiology of disease to future therapies which constitute an important societal application field of biology, including HOX biology. As a corollary, because the use of these technologies is expensive, we have to convince public authorities, governing bodies and funding agencies to maintain significant support for this basic research that fuels applied research.

Do you have regrets about projects which did not bring you satisfaction or about problems you encountered which could not have been solved?

I guess most investigators discontinue projects when they feel that they would lead to a dead-end or to possibly unsolvable issues…. At some point, it is wise to recognize that some questions will not find a valid answer in a realistic trajectory of time, efforts and expenses. I always had a relatively small research team, it was a choice. I always wished to stay in touch with how everyone is managing his/her project. At the maximum, I supervised a group of 10 researchers. All through my career I succeeded in obtaining funding to run my group continuously. Raising funds is challenging, in particular in fundamental developmental biology. Science is more and more competitive. I witnessed this evolution, as it seems to me that the quality of the applications I've been invited to evaluate the last 10 to 15 years continuously increased. Thus, a growing number of projects with already quite a lot of solid preliminary data finally does not reach the "cut off" criteria to become supported. This is extremely alarming!

Part of my research has always been supported by the Life Science Program of NWO (Netherlands Organization for Scientific Research). In addition, I initiated early on, together with Kirstie Lawson, a European research network on Developmental Biology, involving Rosa Beddington, and Margaret Buckingham. I also participated in other European networks, with Denis Duboule and Robb Krumlauf among other participants. These interactive consortia have been extremely exciting. We also took part in a "HOX and TALE network" thanks to a European COST action (European Cooperation in Science and Technology) led by Miguel Torres from 2009 to 2013. These networks have been very profitable to enable meetings within the 'Developmental biology' and 'HOX' communities, share ideas, and give opportunities to PhD students and postdocs to widen their research experience by moving between labs. I also benefited from support of the Netherlands Government (Netherlands Institute for Regenerative Medicine) in a Research Program on Stem cells in Development and Disease directed by Elaine Dzierzak at the Erasmus University in Rotterdam.

As I just mentioned, experimental approaches of biological questions nowadays include high-throughput molecular biological approaches, such as ChIP-seq, RNA-seq, and ATAC-seq, and the subsequent analysis of the data by bioinformatics. For a relatively small research group, establishing collaborations to acquire all this expertise is vital. The Hubrecht Institute in its current state has offered us many opportunities of collaborations, and we did profit by these opportunities in the last years of our *Hox* work.

What does it mean to be a scientist? What advice would you like to offer young people?

What I liked about being a scientist was to be part of a team with shared thoughts, hopes and dreams (and disappointments). But science requires tenacity and commitment. A basic advice: keep your motivation and curiosity intact through trials and difficulties.

Being a scientist also means being free, free to investigate the personally most motivating questions, within the limits of realism. The privilege of being a scientist is accompanied by responsibilities, responsibilities towards students in training and young colleagues, who should be helped in progressing along their career, and responsibilities toward society, since fundamental research is mostly supported by public funding. Societal instances and funding agencies have a right to expect some feed-back and communication on the advance of the work that is being financed.

The essence of scientific research efforts is delivering answers to up to then unanswered questions. But the most fascinating unanswered questions are being asked by other people as well. This is fine, as experimental scientific efforts need to provide reproducible answers. But it bears a notion of competition, competition to be the first to produce an answer and publish it in a more prestigious journal than those that will contribute answers later on. A certain degree of competition between scientists following common ques-

tions is sane, and contributes to excellence. But even if excellence generates prestige, it should not be eagerness to be under the spotlight. In that respect, an advice for young researchers, as complementary to maintaining their motivation intact, is to avoid misplaced elitism. Succumbing to the pressure to reach high impact journals exclusively can be detrimental to scientific process itself.

What's the next step in your scientific adventure?

Research groups are discontinued when the group leader retires or leaves the Hubrecht Institute. But of course the research questions that fascinate investigators will be taken over by the community of developmental biologists. Research scientists contribute to a limited fragment of the endless journey of discovery. In addition, and most importantly, the many talented scientists whom I supervised for a while (Fig. 4) continue to work either in fundamental research or in related activities. I hope I did help young researchers in finding their own way, and this would represent a very valuable accomplishment. I keep contact with some of them, and with colleagues who have become important to me. Thus, no, the adventure is not over!

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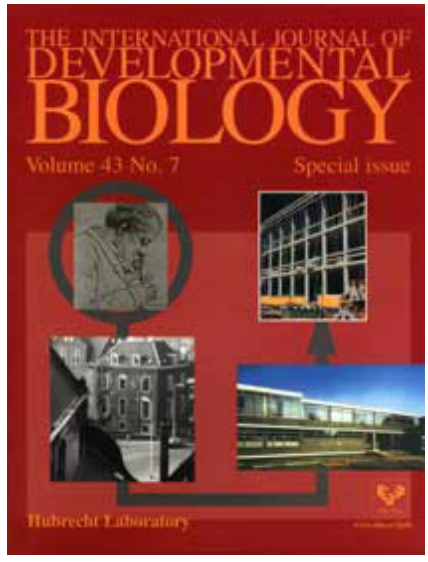

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