Int. J. Dev. Biol. 53: 659-662 (2009)
doi: 10.1387/ijdb.072559mr

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Diffusible gradients are out -

an interview with Lewis Wolpert

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ABSTRACT In 1969, Lewis Wolpert published a paper outlining his new concepts of "pattern formation" and "positional information". He had already published research on the mechanics of cell membranes in amoebae, and a series of classic studies of sea urchin gastrulation with Trygve Gustavson. Wolpert had presented his 1969 paper a year earlier at a Woods Hole conference, where it received a very hostile reception: "I wasn't asked back to America for many years!". But with Francis Crick lining up in support of diffusible morphogen gradients, positional information eventually became established as a guiding principle for research into biological pattern formation. It is now clear that pattern formation is much more complex than could possibly have been imagined in 1969. But Wolpert still believes in positional information, and regards intercalation during regeneration as its best supporting evidence. However, he and others doubt that diffusible morphogen gradients are a plausible mechanism: "Diffusible gradients are too messy", he says. Since his retirement, Lewis Wolpert has remained active as a theoretical biologist and continues to publish in leading journals. He has also campaigned for a greater public understanding of the stigma of depression. He was interviewed at home in London on July 26th, 2007 by Michael Richardson.

KEY WORDS: *positional information, Wolpert L., pattern formation, chick limb, Richardson M.K.*

In other interviews with this journal, you have talked about your early career in civil engineering (Smith, 2000), and have explained your views on pattern formation in the limb (Tickle, 2002). I don't want to cover too much of the same ground again, but it would be useful to start with a brief biography. You grew up in a Jewish family, right?

Oh yes, I am a nice Jewish boy! Most South African Jews have their ancestry in Lithuania — Sydney Brenner[1] and Aaron Klug[2] were Litvacs — but 'Wolpert' is a German name. I liked science and mathematics at school but didn't know what to do as a career. The idea of doing science was so alien to my family that I decided to do civil engineering — mechanical engineering seemed a bit greasy — and I liked the idea of beautiful bridges and that sort of thing.

In South Africa, I was quite involved in communist politics, and politics against the government. At the age of 23, I left the country with a friend of mine and we hitchhiked up Africa for 5 months. I carried a letter from Nelson Mandela introducing me to other militant groups. Then I went to Israel for a year to do engineering, but eventually got a scholarship to Imperial College (London, UK) to do more soil mechanics. By chance, a friend of mine, who knew I wanted to give up engineering, read an article where Swann and Mitchison[3] were looking at the mechanical properties of the cell membrane in relation to cytokinesis. He said: "Lewis, this is what you must do!".

King's and sea urchins

And so I changed directions and went to King's College (London, UK) where my friend was going, and that's where I did a Ph.D. under Danielli[4]. By then I was older than my contemporaries — 4 years older. I think my engineering really helped. And I still

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Notes [1]Sydney Brenner, The Salk Institute, San Diego, California USA, and winner of the Nobel Prize in Physiology or Medicine, 2002. [2] Sir Aaron Klug, formerly director, MRC Laboratory of Molecular Biology, Cambridge, UK, and winner of the 198 Nobel Prize for Chemistry, 1982. [3] Swann and Mitchison, at the University of Cambridge, UK; later at Edinburgh Univeristy, UK (Mitchison and Swann, 1954). [4] James F Danielli, a pioneer in the study of the structure of cell membranes (Danielli, 1962).

Published online: 8 June 2009.

ISSN: Online 1696-3547, Print 0214-6282

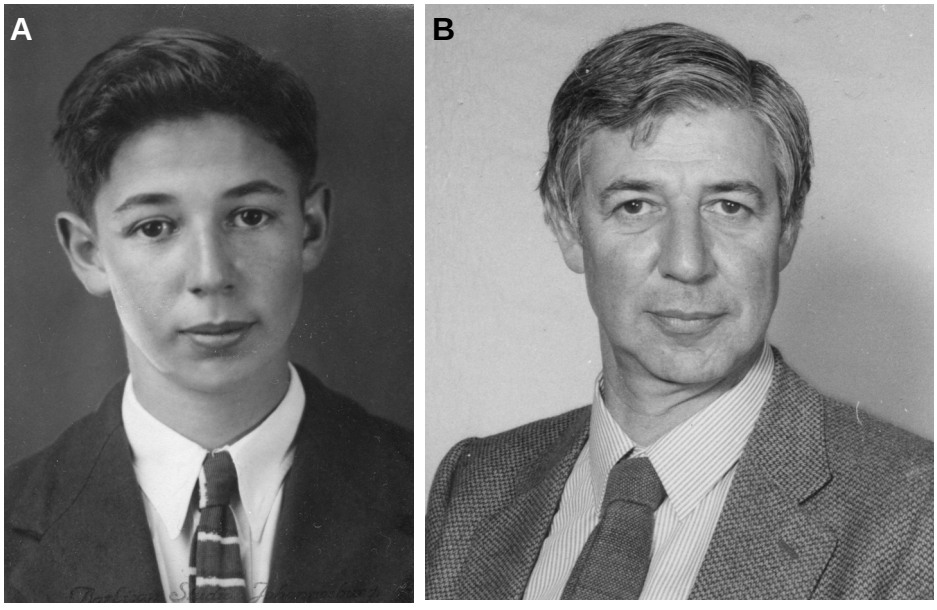

Fig. 1. Lewis Wolpert. (A) *During his school years. Studio photograph, Johannesburg.* **(B)** *Lewis Wolpert in the 1980s. Photos courtesy Lewis Wolpert.*

claim that from my Ph.D., my model for cytokinesis is right, and there is increasing evidence that it is. It is still not generally accepted, although more people are coming round to at least considering it. That was my *astral relaxation theory* which is that the cell rounds up, so there is tension everywhere, and then the asters let the poles relax and so it goes in at the middle (Wolpert, 1960).

After the Ph.D., I got an assistant lectureship at King's (College, London) in zoology; there was no such thing as developmental biology in those days. I started working on sea urchin eggs and also amoebae, because Danielli worked on amoebae. Charles O'Neil was my first Ph.D. student. He did a brilliant Ph.D., isolating the membrane of amoebae, labeling it with a fluorescent antibody then putting it back on the cell and showing that the membrane was fluid. It made a *Nature* paper (O'Neill and Wolpert, 1962) which is very rarely cited!

I worked on sea urchin eggs in Bangor and at a marine station in Scotland. It was a bit lonely there, and I noticed that a lot of other people working on sea urchins went to a marine station in Sweden — Kristineberg. So the next year I went to Kristineberg and that's where I met Trygve Gustafson. He was wonderful, and we started collaborating on sea urchin development (Gustafson and Wolpert, 1961a,b,c, 1962;1963; 1967; Wolpert and Gustafson, 1961a 1961b 1967). We got on extremely well and we had wonderful time. I went there for successive years and then I got married and couldn't go there anymore in the summer. So I moved to *Hydra* and pattern formation.

The French Flag

I was trying to figure out the problem of size-dependent regulation. I started to think of the problem in terms of a French Flag, and then, over a few days, everything fell into place. Because of *Hydra* and sea urchins, I'd got the French Flag problem. From working with sea urchins I realised that pattern formation — in fact, I invented the term pattern formation — you could get the same pattern over a variety of sizes. Halving a sea urchin embryo gave you two smaller sea urchins. We had a little informal group discussing this at King's and I realise that you had the same thing with flags, and I so I asked "how do you make a French flag?" and we came up with gradients.

So this is the crucial step — you published your paper in 1969. Would you say that coming up with your ideas was a 'eureka' moment?

For me it was. There were a set of meetings (Union Internationale des Sciences Biologiques, 1968) that Waddington was having, at Villa Serbelloni on Lake Como, on theoretical biology (1966-69), and Waddington liked me, and I used to go to them. That's the place where I first introduced the French Flag model. And then I was invited to Woods Hole in 1968 and gave one of the Friday night lectures and spoke about gradients and pattern formation. Nobody would speak to me after the lecture or the next day; they hated it so much. And I said to one of my friends the next day, "What is going on?". He

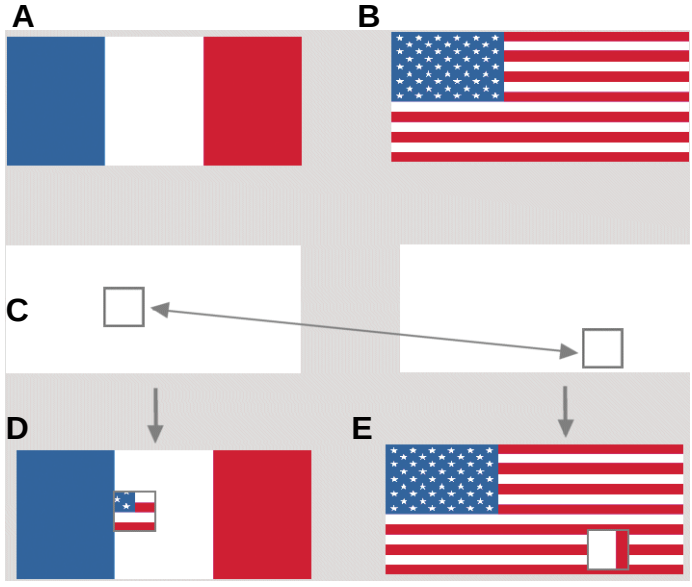

Fig. 2. Wolpert's concept of a universal positional field, illustrated with his *French Flag Model*. *The white rectangles, and the rectangles bounding the flags, represent positional fields; two different genotypes (*fr *and* us*) give rise to the patterns (phenotypes) in* **(A,B)**, *respectively. If reciprocal tissue transplants* **(C)** *are made at an early stage between the two species, the transplanted cells are predicted to develop according to their own genome and developmental history, but to read the positional information in the host positional field. Thus they develop as an island of donor-type pattern that is appropriate to the host positional information* **(D,E)**. *Based on Fig. 5 in (Wolpert, 1969).*

Fig. 3. The Benign Happiness of Lewis Wolpert!. (A) *On safari (1990s). Photo courtesy Lewis Wolpert.* **(B)** *On the beach at Katwijk, The Netherlands, during the workshop "Designing the Bodyplan: Developmental Mechanisms" (June 4th – 8th, 2007). Background (left to right): Leah Winkel (Univeristy of Leiden), Markus Affolter (University of Basel) and Walter Gehring (Univeristy of Basel). Photo courtesy Maximilliano Corredor-Adámez (University of Leiden).*

replied "They are saying: *who the hell do you think you are?*"!

Well, you were a sort of *Young Turk*.

Youngish.

What happened after your lecture?

They wouldn't even talk to me. Fortunately — and I have said this before — Sydney Brenner was at the lecture and he found me, as he says, crying in the water — I was severely depressed. Here was this wonderful idea and nobody was listening. Sydney said: *"Lewis, pay no attention whatsoever. Francis Crick and I think this is very interesting"*. Sydney rescued me from a severe depression. Gradients had a very bad reputation and people were very hostile about it.

Anyhow, then I started on *Hydra* with Gerry Webster and we made a little progress (Webster and Wolpert, 1966). I was at King's for almost 10 years and was promoted up to a readership, and then I got an offer in 1966 to go the Middlesex Hospital Medical School (London, UK) [Now part of University College, London, UK] as professor of biology as applied to medicine.

Chick limbs at the Middlesex

I felt that a Medical School wasn't quite the place for *Hydra*, and so I looked around for something and decided on the chick limb (Tickle, 2002). Amata Hornbruch was my hands, doing all the transplant experiments — and we never had an argument! She was already at the Middlesex Hospital when I got there. We did some *Hydra* to begin with, with Judy Hicklin (Hicklin *et al.*, 1969; Wolpert *et al.*, 1971), and then we moved onto the limb.

Presumably you feel that your diffusible morphogen gradient is vindicated now?

No!

What do you mean?

It's much more complicated. The irony is that a paper will come out shortly in *Cell*, in which Michel Kerszberg and I say that diffusible gradients are out (Kerszberg and Wolpert, 2007). I still believe in positional information, but the idea that you could reliably specify it with a diffusible molecule, we have decided, is out of the question. It must be direct cell-cell contact, a sort of Lawrence and Struhl model for polarity, where one molecule binds on one side of the cell and this precludes binding on the other side (Lawrence *et al.*, 2007). So it has to be something much more reliable than diffusion, and several people have begun to realise that diffusion can't do it. Retinoic acid and *sonic hedgehog* as diffusible morphogens — it is too complicated and too messy.

So, is positional information is still in?

Oh yes, I still believe in positional information. The best evidence for positional information comes from experiments on intercalation. You know, those old experiments on the cockroach leg where you cut out a piece and it intercalates the missing piece. And then regeneration of course…

You mean the urodele experiments where you cut off the limb at different proximodistal levels, and it knows exactly how much to grow back?

Absolutely!

In the 1969 paper, you made a point about developmental fields being small.

Yes, and that made Francis Crick think it was diffusion, you see. It's just not diffusion. Well, that's our line at the moment [laughing].

In your work on pattern formation, you didn't make much of evolutionary implications. In your 1969 article, you constantly talk about universality?

Yes I do. I have not worked on evolution, and I am not good at it. It must be nice to be a creationist because you don't have to do any science and struggle with the diificulty of how things evolved.

BOX 1

LEWIS WOLPERT: SELECTED BIOGRAPHY

1929	Born, South Africa
1961	Ph.D King's College, London
1966	Professor of Biology as Applied to Medicine, Middlesex Hospital Medical School, London
1980	Fellow of the Royal Society
1990	Awarded CBE
1986	Royal Institution Christmas Lecture. *Frankenstein's Quest: Development of Life*

What about the intermediate stages and why were they adaptive? Monkey to human, no problem at all. But how did the cell cycle evolve? I'm not religious, I just find it mind boggling.

So where do the phenotypic differences between species come from?

I think positional information is the same in insects and invertebrates. But it's how cells interpret that information that gives the differences. One of the big influences on me was the *Antennapedia* mutant: a mutation in a single gene turns an antenna into a leg, and when you make mixtures, it looks as though the leg and antenna cells are using the same positional information but are interpreting it differently. And the grafting experiment where they put thigh tissue to a distal place in the chick wing, and it grows into a toe (Saunders and Gasseling, 1959).

It sounds like you really love science.

Oh yes, we had a great time. I had wonderful Ph.D. students — including Denis Summerbell, Nigel Holder, Jim Smith, and David Gingell and others: and wonderful postdocs: Jonathan Slack, Julian Lewis and Cheryll Tickle. It was a wonderful group there, and we all did very well.

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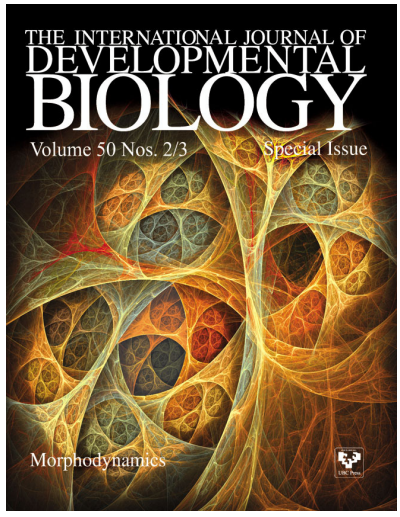

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