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Debatable issues

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ABSTRACT This paper reports a discussion between Antonio García-Bellido and Lewis Wolpert about a number of questions raised by Alain Ghysen. The questions follow, in reverse order, the subjects dealt with in this issue: first the principles (are there unifying principles of development?), then questions dealing with evolution (why are patterns conserved?) and with the homeotic genes (what is their function?), then the cell biology of development (who is controlling actual morphogenesis?), and the generation and evolution of patterns (what makes development so reproducible and how does it change from one species to another?) and finally about the genetics of cell determination and specification (how does a cell measure its position?). Obviously the discussion did not provide any firm answers to any of these questions. Perhaps more importantly, it provides a vivid picture of two contrasting ways of thinking about developmental problems.

KEY WORDS: *pattern formation, morphogenesis, developmental genetics, evolution, cell determination*

Introduction, by A. Ghysen

In preparation for this discussion I had defined a set of questions, all of which are impossible to answer at present. The reader should not, therefore, expect to find firm answers to any of the questions. My aim was not to gather answers or information, but to gather thoughts, or rather to gather ways of thinking. One way of helping students develop a deeper understanding of development is to have insiders tell them how they themselves look at it.

What came out of the discussion, rather than clear ideas about precise problems, is the realization that Antonio García-Bellido and Lewis Wolpert portray the essence of development from two complementary and equally compelling vantage points. I doubt that the views expressed here, with their contrasting emphasis on the abstract and on the real, on the general and on the particular, on the primary causes and on what really happens, might have been more explicitly expressed by any other than García-Bellido and Wolpert. This is because they have spent their entire lifetime pioneering, redefining, pushing these views to the limits, constantly reassessing development in their own terms. There cannot be any unique synthesis of these contrasting views, and any attempt on my part to draw one would be foolish and completely besides the point. It will be the reader's job to draw her/his own one. Yet I trust that stimulating elements will be found in the material provided here.

Most of the problems discussed here are dealt with in more detail and with more background in other papers of this issue; the reader is referred to those for references.

Materials and Methods

The idea of a joint interview of Antonio García-Bellido and Lewis Wolpert originated from Lewis' inability to contribute to this volume, and sadness at this impossibility. I thought that a discussion that I would record and edit might be an interesting way to circumvent the problem. They agreed instantly, and we met on May 5th, 1997, in Madrid.

It soon turned out that, although I had planned to act as a moderator, I could not moderate much, and I came back from our meeting with three 90 min tapes. As I began to transcribe them I realized that we had behaved as civilized persons here and there, but in many cases we ended up all speaking at the same time, trying to outspeak the other two. This was particularly pronounced for the hottest and most interesting issues, when each of us felt very strongly about his point and wanted to express it most forcibly. The end result, of course, is that the soundtrack became totally inaudible.

To convey the flavor of the meeting, here comes a partial transcript of a tiny part of the discussion. Lewis had told me on our way to the meeting place that the major difference between Antonio and him was that Antonio does not pay any attention to the cells, while the previous evening Antonio had told me that the problem with Lewis was that he is not truly interested in the cells. I opened the discussion on the cell biology of development by telling them their respective opinions...

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Antonio García-Bellido: Well, yes, for Lewis the cells are just bricks transparent to signals -but me, all my life has been spent in telling what cells do.

Lewis Wolpert: I came from cell biology, I worked on amoeboid movement for God's sake, I don't know about genes, I know about cells!

A.G.B.: I'm looking to the cells from the point of view of genes, that I confess... which is the only way of giving a personality to a cell.

L.W.: No, proteins and cells! (...) transcription factors are absolutely irrelevant. What do you think gastrulation is?

A.G.B.: It's gene specificity of cell recognition.

L.W.: Oh my God! Listen, I must say that your concept of gastrulation is simply false -gastrulation is a totally mechanical phenomenon.

A.G.B.: Well I think it is totally chemical.

L.W.: No, it is not chemical -I'm sorry, as Newton showed, when something changes shape you have to have forces, and that's what mechanics is. Now, it's no question that changes in cell behavior are determined by which proteins it has, and ultimately that will be determined by genes -genes which make new proteins, which then makes the cell change shape, which generates a force, which brings about gastrulation -it's about forces, Antonio!

A.G.B.: No, you are talking about -I'm talking about primary causes of things.

L.W.: I'm talking about what actually happens.

...and this discussion went on and on, never repetitive, always witty and provoking, and definitely exhausting. They split as they met, excellent friends wondering why the simplest aspects of development sometimes seem so difficult to explain to others... As Galileo said, "you cannot teach anything to anybody, you can only help him discover it by himself".

I should have realized by then that, as exhilarating as the experience was, I could not transmit it -all I could do was to extract a few intriguing or illuminating gems from all the shouting and laughing. In doing this I have tried to use only verbatim excerpts, to preserve as much as possible the spontaneity and authenticity of the exchanges. This, of course, has the disadvantage that the seams are often perceptible, and the text is a bit rough. In the following compte-rendu, "AGB" stands for Antonio García-Bellido, "LW" stands for Lewis Wolpert, and my questions are in bold.

Discussion

The principles of development

The unifying principles of development that we all look for and hope they exist, have such really been found? And are they principles, or remnants of our monophyletic origins?

L.W.: I would say that the principles are really rather few in development. One of the most important principles is that of the enhancer: a modular control element which is only activated by a particular set of interactions or signals, and therefore defines a particular position and time. Another principle is that evolution is lazy, and once it discovers ways of making forms it sticks to them: conservation is a principle. If you look for example at how many classes of intercellular signals there are, they all fall into 5 or 6 classes of protein families, each of which is used in very many different processes... Once you have got the principle, that a particular signal can activate a particular gene, you use it again and again and again. I'm afraid that once we understand precisely how for example *dpp* turns on one of those genes at a particular concentration, or how *eve* is turned on at a particular site because of the various proteins there, that's it -there is nothing else to understand about it, that's the way it is.

As Thoreau put it, "if you are acquainted with the principle, what do you care for a myriad instances and applications?"

A.G.B.: This is true in any science -go to physics: you know the parameters involved, you know the energies involved, you know the constraints involved, and you describe the elements or the association of elements. In biology you have to explain two things: one is the mechanism by which the things happen, which is universal and it would be a principle, and then the particulars, the particular gene doing something at a particular step, and that is not general. But even for the particulars you have another level which is still invariant -you have to define which level you are talking about, because there are levels! The DNA has very few possibilities as DNA of being transferred to brother cells, or of mediating cell interactions -there are many levels between the DNA and the cell interactions, and at each level the system is stuck with those operations or principles that worked well at the beginning. Because you cannot change your opinion when one molecule is talking with another, when one cell is talking to another: you would loose the connectivity. And this is what most mutations do: they are fatal because they are breaking the conversation. So the only way of progressing is just by going to iteration, combination, using again and again modules that happen to be there, because they worked well at the beginning.

But those are the basic features of cell organization, and of transcriptional regulation -is there anything besides this, any principle for development?

L.W.: Practically all animals have separate axes along which development occurs. I would say this is a very important principle. The principle is: it turns out that it's much easier to specify patterns along one axis than on a surface, or even worse in a volume, so initially you make a system that can be specified in 2 dimensions along two separate axes, and you make a third dimension by gastrulating.

A.G.B.: Yes but this is a metaphysical redescription of a phenomenon, an a posteriori -it is not an explanation. Animals have volume, they have polarity, and then you can have as many polar relationships as you can imagine. In fact if you take an animal with an antero-posterior and a dorso-ventral axis, at the moment appendages are growing you are generating a new proximo-distal axis and now there will be further axes perpendicular to this new axis of the appendage. Why should it be simpler to pattern in 2D? It's not that it's mechanically better -it's simply that the system passed through the two dimensions, and the third one was constructed by invaginating, folding, delaminating...

Yet such "principles" are quite different from those in physics -as Yuh-Nung Jan once put it, "in biology we may have, at most, half-principles..."

A.G.B.: A principle in physics is something that accounts for all the observations, whereas in the case of animals, you depend very much on the level of complexity you are describing -so the notion of principle has to be transformed for the biological world. Obviously genes are related to development, but the way they are related is not directly gene and developmental event: we have to consider each level in turn, and the constraints that happen to limit the possibilities at each level, not because of mechanical or physical or energetical reasons -but because the whole biological world is constrained. If you are talking about molecules, there are constraints in molecules: they can do only certain things, not everything possible is there, and in principle they are defined by the limitations to the generality. And now comes the serious question you put just before -are they causally deterministic because it is the only way of doing things, or is there a contingency element, namely that the thing happened to be there...

And what is your answer to that?

A.G.B.: I would say that, from the very beginning, most of biology is contingency. Which does not mean it's chaos: it's constrained by the external world, by physical forces, and by the internal world as well; there are principles in the molecular world, there are principles in the cellular world, there are principles in the organismic world, and there are principles in behavior at the level of systems -namely there are constraints, and I think this is precisely what biology is about, constraints. For example, in order to generate the internal organs, you have to have an invagination sooner or later, to generate a third dimension which actually is a second and a half dimension...

Let's consider the dorso-ventral axis -the genetic system underlying its organization has been at least partly conserved between arthropods and vertebrates, while the axis itself has been inverted. This is most remarkable, but what exactly have we learned from that?

A.G.B.: One thing we are learning, and for me it's absolutely fantastic, is that the apparent diversity we find in forms are just small derivations from common invariant properties. In this particular case something that was considered as a classical dichotomy (between hyponeurians and epineurians) falls into pieces as being an artificial abstraction -at the generative level, both in development and obviously in evolution, we see once again that there is a lot of invariance, and that is something that satisfies many of us. To find invariants in development and evolution is something we could never have thought of, say, 30 years ago.

L.W.: Evolution was lazy, I keep saying, and that's a very important principle.

A.G.B.: I call it lack of imagination -evolution is not original: it's piecing, combining, iterating... It was a tremendous effort to construct the photosynthetic and the energy transduction mechanism, but the moment you have invented them (which were already invented by the procaryotes) you use them as elements that you can combine with others in different ways. Biology then becomes something akin to chemistry because you start knowing which are the properties of the elements. But this can be done only by making a comparative analysis, and this is at the cost of loosing interest in the small details.

The conservation of pattern

A somewhat related question about conserved aspects of development -not only are genes and operations strongly conserved, but patterns themselves seem to be conserved. For example the pattern of expression of the *Hox* genes is strongly conserved, but you might say this is very important for the embryo...

L.W.: I would say that, yes.

yet there are other patterns that do not seem so obviously important, for example the pattern of bristles on the back of the flies, and are nevertheless very strongly conserved?

L.W.: It's an interesting question, but I think I would use as an example one that I know better, the bones in the vertebrate limb. You never get two humeri, or an humerus growing elsewhere -and I'd claim that this is because these elements are not laid down by positional information. We just don't know, but it may be something like a reaction/diffusion, or lateral inhibition, or some way of generating periodic patterns, and that is very difficult to alter. Say, the way the limb develops is by using a mechanism that produces first one element (the humerus), then two, then three and eventually five (fingers), it's very difficult to modify once you get this as a functional system. In principle it's possible, but you would have to make so many changes, that it may not be possible.

A.G.B.: Maybe it's because the system of construction is so integrated -you can change the external appearance of things, like the difference between the wing of a bat and the fin of a whale, but the generative aspect remains the same, because it is very difficult to change that.

L.W.: But you are in difficulty here, Antonio, because it's terribly easy to get six digits, lots of people have six digits, the question is why don't they have six different digits, and that maybe is because you don't have enough *Hox* gene variation to give you the extra identity for the sixth digit.

A.G.B.: You get polydactyly by mutation, accident or whatever, but it is always a variation on a five-finger pattern; and when you have a reduced number of fingers like in horses, it is also a variation of the same pattern. The system re-uses again and again the normal mechanism by which the fingers are formed -the whole evolutionary history is behind the decision to form five fingers, you cannot change that, the system is caught.

But certainly in the case of the sense organs in the fly it is not too difficult to eliminate some, or add some; yet the earliest set of sense organs is nearly identical in the fly and in the grasshopper embryos, although each of them will assume a different function in the two animals...

A.G.B.: Your question relates to the following: why do incipient organs are the way they are? Is it because they were selected? Maybe not, but either way, the first organs are the first ones. Period. And once they are there you can only modulate, not because there is any causal reason but because the system is stuck and you cannot change it.

L.W.: I would take a slightly different view. I think the phylotypic stage, for example, which is so strongly conserved for all vertebrates, is the stage in which you are laying down positional identity along the axes -you can change the way you get there, and what you'll do afterwards, but you cannot change that stage, because that is when you are putting down your coordinate system. So I would think that there are some crucial steps in development that are conserved because of the mechanism, because of the essence of the step itself.

A.G.B.: But the fact that there is regularity does not mean that there is a very profound cause for the regularity -this is my point. We are embedded in the description and in the classification and we say: this is very general, there must be a fundamental principle that explains the generality -but maybe not, maybe there is nothing special to be explained there: it just happened to be there, and happened to have lots of progeny...

Developmental vs molecular function: the case of the Hox genes

According to your view, Lewis, the function of Hox genes would be to record positional values. On the other hand Antonio would say that their function is to control target genes. So we have this paradox that their developmental function seems not to change at all, yet their molecular function-the battery of genes that they control -changes from one organism to the other...

L.W.: The essence of development is to make cells different from each other. As far as the antero-posterior axis is concerned, the *Hox* genes are giving positional identities, and once you've got positional identities in an ordered manner then your hands are free, you can do what you want at all those positions because you can now change the downstream targets.

That means that knowing the molecular function of a gene may tell you very little about -

L.W.: Nothing! The downstream targets are what matters.

A.G.B.: But it depends on which is the level where you measure it. For example, it has been found that the same enhancer regions are recognized by the products of the *labial* gene of *Drosophila*, and the *Hox3* gene, meaning that you retain the connectivity between protein and DNA sequence all the way from insects to mammals. Why? Because the real constraint is molecular recognition. Molecular recognition holds the whole biology together. And now this is making operations, what you call positional information or defining territories of cells, and once you have it you retain it throughout evolution because of the inertia.

So what is it exactly that is conserved in evolution?

A.G.B.: The operation!

And what is the operation that the *Hox* genes are involved in?

A.G.B.: Well, several. One of them is to define territories of cells, i.e., territories as distinct from other territories. So the primary function of the homeotic genes is to talk with other genes. But it's not simply a dialogue between one gene and another, it's a battery of genes, right? And the function of a gene is defined at the beginning by the battery that responds to that gene, whatever it is, making the territory number 3 for example. Now you can start changing the elements downstream and you still retain the specification of the position number 3 because the change is piecemeal, this is my point. You are trapped by the first function, or operation, by which a syntagm came to functional existence, by the first function it came to be used for.

Would you agree with Lewis then, that the function of the *Hox* genes is to record positional value?

A.G.B.: No, I would say that their function is to talk to other genes.

L.W.: I think you are making it more complex than necessary, and that you loose a great deal if you don't want to use the *Hox* genes for positional identity.

A.G.B.: I am interested in the generative aspect of things, you are more interested in the end result of things.

L.W.: No, no, I think that an essential feature for generating patterns in any system is to provide positional identity, and by doing that, you make the cells different...

Let's shift to another aspect of the *Hox* genes, their role in limb development

L.W.: To be absolutely honest, the relationship between the *Hox* genes and limb morphology is quite obscure... There are very few situations in which you work in 3D. Bones are essentially 2D, because it is usually the perichondrium and the periosteum that determine the shape of the bones. The curious exception is the limb, where you do things in 3D, with three axes: antero-posterior, dorso-ventral, proximo-distal. So, about the *Hox* genes in the vertebrate limB.: one answer could be that in vertebrates the *Hox* genes are involved essentially in mesodermal tissue. In the insect limb it's not the mesoderm that is doing the patterning -maybe that's why you don't use the *Hox* genes to pattern insect appendages? One of the things we did many years ago was to show that the shape of the end of the humerus, for example, or of the wrist, is quite normal even if you don't have the distal elements, so they develop totally autonomously. This means that there must be an extremely fine control of skeletal morphogenesis -how do you do that? This is about the interpretation of positional information -just think of the wonderful example of the panda, where a wrist bone becomes an additional finger... You can locally modify everything -that's what those *Hox* genes are for.

But in the limb you just said you don't know what they are for?

L.W.: No, I know what they are for, it's for specifying all those fine details; what I don't know is how they operate.

A.G.B.: When you go to the appendages in insects, however, even though you are not using the *Hox* genes, the surprising thing is that, later on, you have *hh* and *dpp* operating again as cell

Fig. 1. A typical case of hereditary nose shape: the Bourbon family.

communicators, making the insect limb superficially similar to the vertebrate limb.

L.W.: Oh no! I think there's much besides this -what you say is superficial I say is very deep- there's no other way of doing it.

A.G.B.: This comes to the profound problem of homology vs analogy. *hh* and *dpp* are signaling molecules, they do not specify things, and if you have a finite number of signaling devices you are bound to use them again and again. But I agree that it's very difficult to answer these questions: you can just make guesses...

Gene regulation and morphogenesis

Let us shift to the cell biology of development -we do not seem to have any idea about why a cell has its particular shape...

L.W.: First let me emphasize that the complexity of development is really about the complexity of the cell. Cells are much more complicated than embryos -what's going on in a cell is much more complicated than what's going on between cells in an embryo. And morphogenesis, which is about changes in form, is largely about cell mechanics, and the types of forces that cells generate are very few: tensions, occasionally extensions, and changes of neighbors. It's true that we don't have a good connection yet between the patterning and the morphogenesis. But I think you are a bit unfair: people studying cell shape, mitosis, outgrowth of growth cones, they're doing better

Which brings me to the next question -there is a very obvious gap between the developmental controls (selector genes), and what cells actually do, like for example defining the shape of a bone...

A.G.B.: The real dimensions -the species-specific dimensions. There are dramatic changes in the shape of the wing in the genus *Drosophila*

between species, yes, but neither in mouse nor in fish nor in fly have people come up with mutations that change the shape of one part (other than messing it: notching the wing, roughening the eye, fusing leg segments...).

L.W.: Local transformations -you wonder where the mutations are, where the genes that control the fine structure of the shapes of bones are, for example. I don't think anyone knows yet. We all three have very different noses -which are the genes that are controlling that? It must be a combination of genes that are changing the shape of your nose... The question is, does it require many genes, or just very few will do it?

It could be rewarding to study the genetics of nose shape (Fig. 1), or other familiar traits. But to come back to flies -when you look at the leg of a fly, every single joint is incredibly subtle. How is that controlled?

A.G.B.: There's not such a level of control, it is a fallacy. There's nobody controlling the things in this detail. The actual shape of a bone, or of a fly leg, is the result of a series of genes doing vulgar things. As Lewis said, it's a combination of genes working in many other places in the fly, and mutations in any element of the combination will perturb the whole process. The actual solutions of the combinatorial define the shapes of different species -what I call the real dimensions, a particular collection of allelic states of particular genes. Your question cannot be asked unless you define it already by the mutation -you cannot go to the continuum of an animal, and start asking for things in the middle of the continuum, because it's not in the logic of the animal. Maybe there are more genes involved in the later steps, maybe defining the detailed shape of things requires more gene activity and therefore it's more difficult to pinpoint anything?

L.W.: but that's not saying anything, Antonio, that's handwaving -we want to know the mechanism, which are the genes that are controlling that this (bone) has this little bump here.

A.G.B.: Sure it is hand-waving, but what is the alternative? Maybe the question is a wrong question, and therefore the answer is, I don't know. Why is it raining now? I don't know. It's such a chaos to understand that...

L.W.: I don't want to think development is like the weather, please, I'd like you to withdraw it...

A.G.B.: (laughing): all right -well, it was an analogy...

Feedback and redundancy in development

Lewis, you mentioned earlier that the types of forces that cells generate are very few, and that small changes in any of them can have profound influences on the end result. One would think, then, that there should be lots of feedback mechanisms, to ensure reproducibility...

L.W.: Very little feedback at all -one of the most remarkable features about development is the virtual total absence of feedback.

A.G.B.: but there is feedback! Development has lots of regulation, and that is a form of feedback. Most of developmental operation involve counteracting forces, they are done by antagonisms. The way the HLH products work is by titrating each other!

L.W.: Titration is not feedback: a threshold is not a thermostat. Negative feedback has a well-defined classical meaning: you actually have to measure something, and then if you have too much you make less, and if you have too little you make more. There is no feedback in development, nor even in the regulation of developmental genes: if you put extra copies of bicoid, you make more bicoid proteins

A.G.B.: And what happens in the end? The embryo is exactly the same, the mistake is corrected later on because there is regulation...

L.W.: But developmental regulation is also not feedback: you don't act back on the original operation, you compensate later. And I think that the answer is that there are two ways of getting precision -either you use a negative feedback like a thermostat, or you have multiple ways of doing the same thing. And in general the way development works is by multiple processes, what Waddington called canalization. And that's why you think you have redundancy. In mice, there are many genes that you knock out and you don't see a phenotype, and one concludes that they are redundant. I say, have you taken your mice to the opera? Can they still tell Wagner from Mozart? It turns out from simple population genetics that, in order to pick up a 1% selective advantage -which is evolutionarily very significant -you would have to look at something like 10,000 mice... So you've got to be careful.

But how do you select for redundant mechanisms? What could drive the multiplication of processes or mechanisms doing the same thing?

L.W.: There is a second aspect to multiple parallel control: reliability. The real thing that embryos have to do is to be reliable, that's the most important thing in their lives, and the simplest way of getting reliability, as rocket engineers know, is to put in extra components, to do things in multiple ways, in case one fails... for example, ensuring the development in adverse conditions may lead to the multiplication of processes. Take one of my favorite examples: one of those genes they didn't know in yeast, they finally found out that it protects it at pH 4.5... Maynard-Smith had this metaphor, that it is an advantage to have a lightning conductor even if lightning strikes your house one in a thousand times. So if in one thousand generations there is one exposure to some rare condition, such as pH 4.5, that affects a particular developmental process, it would be an advantage to have an alternative system that can take over.

A.G.B.: But be careful about reliability -the system has no perception for possible failures, the system is not preparing itself for the adverse events, the system is doing things... it cannot know what reliance is, before it has been exposed... that's teleology, that's when you look from the end, a posteriori. A lot of the exercise has been done looking from the end, which is nonsense, but if you look from the beginning, at the beginning you are increasing a net of interactions, the genes would do sloppy things, just to make an appendage and so on, and then the system is getting more and more variation, and gets into more and more details, and later on functions which were devoted to something else start participating -nothing to do to ensure anything (they don't know what to ensure), nor in preparation for something that may happen... And it happens that by doing this, you are creating redundancy. It is in the logic of the system to accumulate more and more parallel processes, with the consequence that once you perturb one, there's another one that does the thing -approximately. But from there you cannot conclude that with one single mechanism you have enough. The accumulation of parallel processes increases precision because you get more qualification by doing that, because you are titrating amounts...

L.W.: I agree: if you wind back -this is never discussed, but if we could wind back to early animals, you would find their development was very unreliable, they were messy, very susceptible to any environmental perturbation. And redundancy is really about precision, more than reliability. My line is that if you knock out a gene, and you have no phenotype, that gene is really important. Not at all because there may be a second copy of it somewhere, to ensure against a loss of the first one -that would be an a posteriori explanation, as Antonio keeps saying. But because it is involved in a process that is so important for the embryo that it is controlled very precisely.

Speciation

Given the nearly infinite possibilities of modulation and variation of existing programmes, how do species become fixed for several million years on average?

L.W.: For me it would be entirely about selection and adaptation to the environment... I know it's a boring answer, but I don't have a better one. If you are happy, why change? True that you have new mutations all the time, but if they are not adaptive, good bye!

You mean that mice have remained mice for this long time because there is some subtle selective pressure to keep them mice?

L.W.: Absolutely! Why change, if you are doing well? You only change in order to survive, you only change if you do better. Why should it stay so constant for so long -I don't know, the answer is in population genetics, it's not interesting developmentally. The great revolutions of the early Cambrian, the rise of new animal forms, that could happen because development was very sloppy then, as we discussed earlier. They could survive, but their competition was lousy. I think that canalization and precision and reliability have become essential features of development. And I think that evolution is mostly over now, because things are so canalized. I would bet that if we could come back in 100 million years we would find pretty much the same animals as we know today.

I know you have a very different view of evolution, Antonio...

A.G.B.: In my view evolution is made by propositions. There is punctuated equilibrium: you have a long stasis, and then suddenly there are changes, and there are two explanations for it. One is that there is a change in the world, to which the species responds and adjusts, the Darwinian view of evolution. The other is that the changes are internally driven: small population, genetic drift, and then you have to accommodate for shifts of the average in one direction: the other parts have to co-evolve and to co-adapt, and bang! a series of new propositions. This co-evolution is achieved by small changes but very fast. The system is not in equilibrium then, but most of the changes are not even perceived. There is enough tolerance to accept short-lived transitional forms, and that leads you to new solutions to the combinatorial, to new species. When you have an extinction, the competition pressure diminishes dramatically, the tolerance increases, and this is the reason we have explosions, which are proportional to the amount of species that disappear. The notion of small differential selection in the Darwinian sense is fine for some subtle details, but in the early Cambrian, or in the later bursts of radiations, it's just a tremendous amount of new propositions.

And why do you think there have been so many new life forms appearing in the Cambrian times, and so few since then (but see Fig. 2)? Just the fact that the competition pressure was very low does not really explain the extraordinary diversity of forms that appeared in such a short time...

A.G.B.: Obviously we don't know, but one thing we know is that these animals can be decomposed in genetic operations for making axes, for making iterations, for making terminal differentiation, we have fossils which are chimaeras with parts that could be assigned to a mollusc and other parts to an arthropod, so my feeling is that these genetic operations were just assembled as propositions, and there were very many because there was no way of defining what propositions were in the general world of the pre-cambrian. So it is an explosion, in a sense. But from then on you can only modulate, getting in further and further detail, more and more adaptive in the real sense. And the new radiations that appear after each geological extinction are not requestioning the whole procedure: they are branches within branches within branches, so that you end up increasing in diversity, not in complexity, and this diversity has less and less taxonomic value.

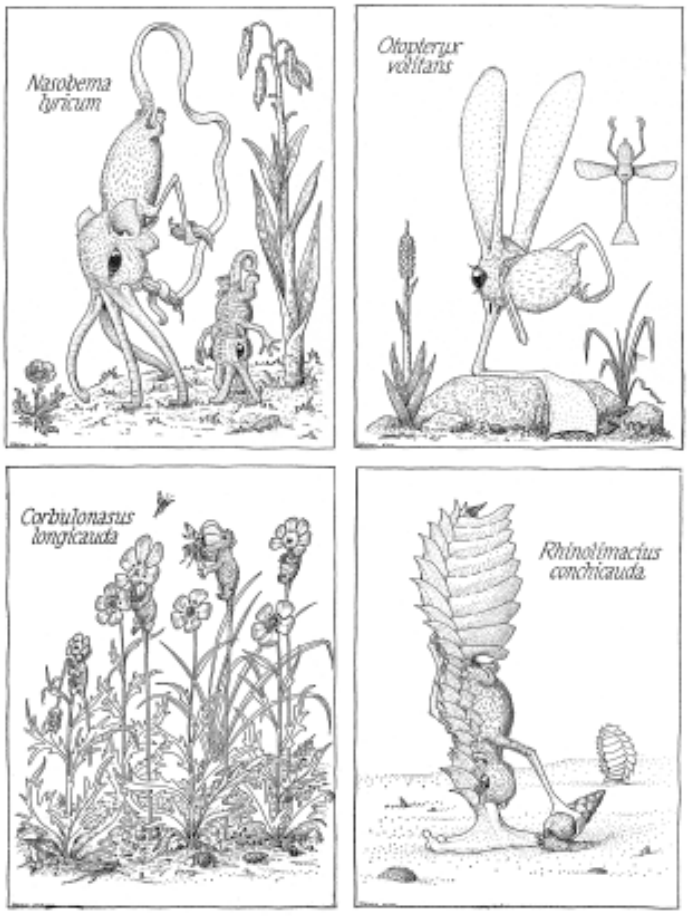

Fig. 2. Four among the many species formed during the spectacular radiation of the rhinogrades, a new order of mammals that diversified in the Hi-iay islands. *Plates III, VIII, X and XIV from the "Anatomie et biologie des Rhinogrades, un nouvel ordre de mammifères", H. Stümpke, Masson et Cie eds, Paris, 1962. Reproduced with permission.*

Cell determination and positional specification

A similar set of bHLH genes is involved in neural determination in flies and vertebrates, suggesting that it has been associated with that function for 600 million years -why such a strong link?

A.G.B.: There is nothing that can substitute for it! It is very complicated to change, too many things at the same time, so you stick to it. Molecular recognition holds the whole thing together: there is a function, to activate the downstream nervous genes, and you cannot change overnight the relationship. Nothing is against it, why should it change?

L.W.: I agree -if it didn't break, why fix it? Or in other words, if you have a way of making neurons, why change it? I think it's no mystery: it just reflects ancestry, like in the case of the *Hox* genes. But I think neurons and muscles are the exception -I think you won't find it elsewhere. For most cell types there is not a single key gene that determines them. I have a view of cell differentiation: that it is contingent, and that it's a combination of transcription factors with no particular logic.

It is usually said that there may be about two hundred cell types, but one can further subdivide, e.g., there are many different types of neurons, and one might even argue that each neuron is unique...

A.G.B.: But not different in a qualitative sense. Up to a certain degree, differences are qualitative, and the rest is quantity, and this is much more difficult to analyze. But you see, the issue of development and morphogenesis and size control is a quantitative problem, and we don't have the mentality to deal with it. This is the reason why so few people work on that...

L.W.: There is good evidence in many systems that there is some sort of gradient, or quantitative variation, between cells. That allows you to go between particular values, it allows intercalation, and this is also true in regeneration. But we do not know what these quantities are. I think if I had to take a guess, it would be something like the Notch-Delta system, something like how many receptors you have -but we don't know.

A.G.B.: intercalation is crucial -from any level, not just from the boundaries! You intercalate from any level, anywhere in the system, and you fill up -until you get the entelechia condition.

This brings us to the issue of positional information: there is evidence in many systems that cells must be able to remember their positional values, how would they do that?

L.W.: That's the distinction between setting it up and remembering it. If you take a cockroach leg, each one of those cells remembers its position -it's got to be recorded somehow. But we have no idea about how you record it. Maybe it's by the number of receptors on the surface, maybe by genes, or it might be the amount of calcium...

A.G.B.: But the value changes with time -the cell is not remembering the actual scalar value, it is changing all the time. In the regeneration experiment you have a reference, you regenerate according to whatever is presenting you values, but if you isolate cells -I did the experiment many years ago -it is lost. When cells remain together with other cells they don't have to remember -they have a value which is constantly actualized

L.W.: The value is the memory!

How are those values, whatever they are, used, or read? You mentioned once the case of the feather-by-feather specification, which puts a heavy load on the cell's capability to distinguish different thresholds...

L.W.: We don't have a simple answer to that, but any bird person would tell you that every feather is distinct. What makes them different? Maybe different Hox genes, different combinations of Hox genes being active in those cells... It has to be something like that. There is no evidence for that, but no one has ever looked at feather patterns in relation to Hox genes. Take your vertebrae, look at the subtle differences between them, you don't think it's the *Hox* genes? In addition, cells can discriminate thresholds -let's take the case of the butterfly wing patterns, which are enormously varied, you can have anything you like, it looks like they read lots of different thresholds.

Personal questions

My last two questions are more personal... What would you work on, if you were to begin a PhD next year?

L.W.: One of the subjects I would work on, is the molecular basis for the quantitative differences between cells... probably on the amphibian limb. I mean somebody really ought to go in there. It's a straight molecular question, and I would probably try to fractionate membranes, seeking quantitative differences, or try to make educated guesses and maybe look at Notch and Delta, that class of things on the cell surface... And then there is a completely different area I would work on...

That's two PhDs?

L.W.: I want a second one, in case I cannot find the right supervisor... The second one, on which I may work in my old age, is growth in vertebrates. I would need to understand what determines the number of nephrons in a kidney -I don't even know how to pose the question, but that is what I would work on. Experimental morphology. Because we really don't understand much about the programming of growth, other than that it is hideously complicated.

A.G.B.: For a PhD, I would go for comparative DNA -I would go phylogenetic, taking probes of homologous genes to *Drosophila* and see how they are expressed, in different patterns, at different moments of development -what is called subrogate genetics. I want to understand how the connectivity is changing, what is the logic of variation.

And the last question: what does each of you think is the most important contribution of the other?

L.W.: (both laugh) We don't think either has made one! Seriously now -first of all, compartments and selector genes is what Antonio will always be known for -he changed the whole image...

A.G.B.: The thing I appreciate most in Lewis is his way of putting the question clear -how dimensions are transferred into specific cell types. He brought our attention on how much position plays a role in development -although I would play with his wording by saying that it's not a question so much of positional information as of informational position!

Acknowledgments

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