

The genetic logic of Antonio García-Bellido

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This is an offer that cannot be refused: to put in writing what I have wanted to say about the wonderful world of development and evolution without being overwhelmed by the knowledge and irrefutable logic which pours enthusiastically out of Antonio García-Bellido, in head-to-head conversation. The opportunity to tell a few personal anecdotes about my scientific life with Antonio also cannot be passed by.

My first encounter with Antonio, sometime in the mid-1970's, was as one of the audience listening to him deliver a paper on *Drosophila* development of which I did not understand a word. However, my instincts told me that if any sense was to be made of multicellular development then this was the man who was going to do it. Aurally, I couldn't make the crucial distinction between metathorax and mesothorax as delivered at high speed in Antonio's then heavily accented English diction, yet still I felt a breath of fresh air was sweeping away the old terminology. No more pilpul on determination, specification, commitment, induction, differentiation and that whole rag-bag of terms that turned the study of development into Talmudic arguments over definitions: instead I woke up to the clarity of compartments, boundaries, cell autonomy and selector genes. This cellular event occurs now and here because this gene operates here and now. Developmental genetics was transformed overnight.

Being in Cambridge for many years I could not escape the excitement generated by the new discoveries in *Drosophila* developmental genetics. I felt at that time that some important link could be made between the new discoveries on the redundancy and 'turnover' in eukaryotic genomes and the evolution and operation

of the genetic circuitry involved with developmental systems. Attempts to forge a new synthesis between evolution and development were discussed (rather prematurely as it turned out), at two infamous gatherings in the early 1980's: one at Dahlem, Berlin and the other at Chicago, USA. These were interdisciplinary meetings in which no one person seemed to have the wherewithall or patience to comprehend the biological philosophy of any other. Antonio, as expected by now, was in the thick of it at Dahlem, but I had the impression that his words were falling largely on deaf ears. As has been said of Edgar Varèse, the avant-garde American composer: it was not so much that he was ahead of his time, but that most people were behind theirs.

I remember one particularly revealing contrast in approach at Dahlem which encapsulates the gulf between the hardwired genetics of Antonio and the more soft focussed pursuits of some other biologists who were looking for developmental rules of organization that were supposedly beyond the reach of the genes. This latter group seemed to rely on the term 'epigenetics' to signify 'organizing principles' governing ontogeny which emerge more from the laws of physics and chemistry as they apply to membrane-bound cells, than to genes. It is not possible to be too precise over how a structured organism is supposed to emerge from an unstructured fertilized egg as it rolls around the epigenetic landscape. Nevertheless, the approach, in its extreme manifestation, is in sharp contrast to the alternative approach which is summed up by the word 'epistasis'. This latter term is an ugly sort of word but it is intended to describe the important point, accepted by most everyone today, that genes (or more accurately their products) interact

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one with another. Thus, the emergent three-dimensional structure of an organism, in all its species-specific glory, is nothing but the sequential assemblage of molecules which physically interact, and the order, quality and quantity of such interactions reflects the largely haphazard course of evolution. Hence, there is no 'master plan' of construction lying somewhere beyond the genes or even amongst the genes: rather variation in bodies is a direct reflection of changes in local intermolecular interactions which were consequential on molecular variation in regulatory elements and genes. Once we have grasped this, then the central concept, developed over the years by Antonio and others (for recent review see Arnone and Davidson, 1997), that the phylogeny and ontogeny of biological complexity is a result of ever-changing combinatorial permutations of rather small numbers of locally acting and autonomous modular components -at all levels of biological organization- is inescapable. All that is required for novelty to succeed (and here I paraphrase Antonio) is for the new combinatorial arrangement to be acceptable to the rest of the players in the internal cellular and intercellular arena and not necessarily judged by the external forces that govern the processes of adaptation and natural selection.

At that time this was music to my ears for it was becoming clear to genome pushers such as myself that the internal processes of genomic turnover (unequal crossing over, gene conversion, slippage, transposition, etc.) could initiate and spread novel genetic combinations, including those involved with regulatory networks. The dynamics of spread caused by such non-Mendelian systems led us to believe that the main criterion for 'acceptability' of a novelty would be the maintenance of internal consistency regarding the construction of the organism. 'Acceptability' might not be too difficult to achieve given the high levels of tolerance associated with genetic and functionally redundant systems.

Well, all that was a mouthful which requires further clarification and a short interlude. Hence, I cannot leave the Dahlem Conference without relating how Antonio's contribution to my well-being was not just scientific but personal as well. One evening he, I and Stuart Kauffman set off down Actionstrasse in West Berlin to have a few beers and wash down, amongst other things, the cloying effects of Stuart's theoretical models of genes and development. We were so busy talking (comparing salaries, I believe, at that time) that we did not pay much attention to the strange fact that the bar we eventually entered had steel doors through which we were not allowed to pass without some extravagant entrance fee. Once inside the dingy bar, we each received the attentions of some wispy girls who asked us to buy them drinks. We cheerfully accepted this spontaneous seduction until we realized their price! Being coy and naive, I wasn't too sure how to handle 'my' girl but I distinctly noticed (even if Antonio cannot remember this!) that Antonio whispered something into the ear of 'his' girl that made her get off his shoulder and beat a hasty retreat! I've never had the courage to ask what he said to cause such alarm. Eventually, we managed to sit unaccompanied discussing, for the whole of two minutes, compartments, Boolean algebra and molecular drive with occasional glances at the blue movies on the wall and at the more suave techniques of a couple of Russian officers. But, inevitably, the girls returned with their demands and with a rather large man. Things were getting desperately out of hand until Antonio took a hand. With one well-timed and apposite outburst he put the surrounding personelle in their place who, in some unaccustomed state of shock, were quite

relieved to open the steel doors and get us, and that Spanish man, out of the place.

After this Dahlem meeting I knew that life, intellectually and bodily, was safe with Antonio. Within a couple of years I was on sabbatical with him in Madrid, along with Rolf Nöthiger, and Antonio and I began the long process of mutual agreement and disagreement over the why's and wherefore's of biological life. By then, Dick Flavell and I had published our small piece on 'molecular coevolution' (Dover and Flavell, 1984) which struck an immediate chord in Antonio, given his extensive thinking on the internal 'logic' of the genetic machinery and its evolutionary self-consistency, as alluded to above. He recognized that multicellular organisms were not locked into one gigantic, frozen network of interacting molecules (or modules). On the contrary, the autonomous nature of modules and their very local activities meant that internal flexibility was the name of the game: new constructions (e.g., legs in place of antennae in *Drosophila*) could arise without major breakdown in the total 'network' which was meant to govern development. What the concept of 'molecular coevolution' proposed was how any such combinatorial novelty might be tolerated and spread in a population as a consequence of genetic redundancy and genomic turnover. Initially, the origination of new combinatorial permutations and the mechanism of their subsequent spread, with the passing of the generations, involve a process (molecular drive) which is operationally distinct from natural selection and adaptation. As Antonio has repeatedly emphasized in private, any altered organism, in its novel yet internally consistent ontogenetic mode, probably 'doesn't give a damn about the environment': the relationship between the organism and the environment is much looser than is assumed under a strictly Darwinian process of selective honing and refining (García-Bellido, 1997).

I say Amen to that. Naturally, selection does have a say in what goes on, but as Dick Flavell and I tried to point out, its role is as much to do with ensuring that interacting modules co-evolve one with another, as ensuring that organisms succeed according to their goodness of fit to external niches. In other words, as Antonio recognized at that time, life is about molecular interactions and that there is nothing beyond such interactions. If new sets of interactions arise, as a consequence of genomic turnover and modular autonomy, then life can continue so long as the consenting molecules, involved with phenotypic functions, are happy one with another. I personally saw a role for selection in ensuring the quality of this 'happiness' but, nevertheless, the central Antonian concept that we have to dissect the genetic and modular rules of construction, if we are to understand the seemingly haphazard diversity of life, was an important paradigm shift.

I do have some differences from Antonio, however, which I want to pursue. One difference revolves around the issue of how do we proceed, pragmatically, to dissect the genetic processes that govern development and to understand their evolution. We both fundamentally agree that this requires a radical move away from the single-species, synchronic approach to the comparative, multispecies, diachronic approach. In other words, we could potentially know everything we might wish to know about how the species *D. melanogaster* puts itself together, yet we would, in a deeper sort of sense, not know or understand anything. We would be ignorant of how and why it got to be that way. The only method available to biologists to explore deeper into once-forbidden questions of 'why' is to examine and compare the same genetic operations and

transformation rules in other forms of life. Again, I am self-consciously aware that this diachronic approach, is now generally accepted, but in the critical years when Antonio was pressing for this approach there was inertia and disinterest afoot. For example, I recall the comments of a reviewer on one of my grant applications to examine the phenomenon of molecular coevolution between the RNA Pol I machinery and the multiple rDNA promoters in the genus *Drosophila*: a study that could only be done, given the central phenomenon of interspecific incompatibilities between Pol I and promoters, through the examination of several species (or at least two, as I had proposed). The referee made the comment (what she or he no doubt considered a valid point), that it was premature to explore two unknown paths at once. The irony being that, yes, each path in itself and in isolation was unknowable, but if explored together some light might be shed on the problem in hand. Needless to say, I had to revise the application and promise to stick with *D. melanogaster*. I took the money and ran, with several species in tow!

For Antonio, there was never any doubt about the power and the critical necessity for the diachronic approach; not just to uncover, as in my case, the changing nature of the molecular basis by which molecules interact, leading to the eventual establishment of species-specific modes of ontogeny, but to understand the deep 'generative rules' that govern development across all life forms. There is clearly a close parallel here with the Chomskian view of the process of language acquisition in infants which is considered to be consequential on the establishment, in the human species, of a genetically governed universal grammar. Antonio has developed the important idea that there are universal genetic operations and developmental processes (his syntagmata) that are widely shared throughout life and which represent the fundamental 'genetic logic' by which cellular and intercellular life can proceed. However, such syntagmata are not a recipe for evolutionary conservation and inflexibility, for diversity arises from the combinatorial potential of subsets of syntagmata operating in different cell and tissue types. Hence, given the universal nature of, for example, processes of cell signaling and signal transduction, ribosome assembly, readout of the genetic code, axes polarity in development, sex determination pathways etc., it is operationally logical to attempt to uncover the detailed nature of such phenomena through a wide species comparison. This does not mean, for example, that we should ascribe to the view, as once announced in *Nature*, that the homeobox is the Rosetta Stone of biology -nevertheless the quite remarkable discovery of *Hox* gene clusters across major anatomical forms of animals (dating back to the Cambrian fauna) justifies the philosophy of 'universals' and the operational approach of wide species comparisons.

So where's the catch? Is this paradigm shift, in which Antonio played a central and forceful role, not enough for the modern analytical approach to biological understanding? Should not the crucial search for the universal 'transformation rules', which govern the transition from one level of organization to another, as we ascend the ladder of complexity during ontogeny, be the central goal of our experimental pursuits and intellectual curiosity?

My personal answer to these questions is both yes and no. We cannot deny that autonomous and locally acting modular units exist at all levels of organization from genes to syntagmata and that complexity and diversity are a consequence of combinatorial

flexibility. Nor can we deny that some such molecular interactions are very ancient and relatively invariant (although I would not go so far to say that they reflect the 'laws' of biology). My concern revolves more around the complementary question of how all such novel combinations, whenever and wherever they may arise, eventually become established in a population of organisms, leading eventually to such a population becoming biologically distinct and incompatible with other differentiated populations. It might be worth saying, even in this volume of distinguished contributions, that, for example, *D. melanogaster* does not consist of one gene, on one chromosome, in one individual, floating in a vacuum. It is not natural, and can be seriously misleading, to consider a gene mutation in such a simplified and unreal scenario which ignores the population and inter-generation dimension. If novel, modular combinations are to be established, following on from genetic mutations, then their success or otherwise has to embrace the spread of the novelty through a population, with the passing of the generations. How does the multitude of new replace the multitude of old?

This is not the place to enter into a lengthy exposition on how biological evolution has learned the trick of changing the workings of an aeroplane whilst the thing is flying in the air. Nevertheless, it is clear that Antonio's lifelong theme, that evolutionary success is dependent as much on 'internal consistency' as on 'external adaptation' governed by natural selection, is an important part of the solution to this conundrum. In other words, although there are limited degrees of freedom governing the extent of permissible combinatorial permutations, nevertheless some new combinations do arise and evolve. How and why are they preferred? And how, experimentally, do we begin to dissect those very early events which represent the thin edge of the wedge, before the novelty has successfully spread in time and space and lead to the differentiation of a new form of life.

It is my belief that this sort of question can only be understood through the diachronic, comparative approach of closely-related species. It is only through the detailed examination of the molecular nuts-and-bolts of modular interactions in sister species that we can understand how internal consistency of cellular and development functions might have been maintained, throughout a period of evolutionary transition. Wide species comparisons can trace back the heritage of ancient genetic processes that our closely-related species need to contend with, but such wide comparisons are insufficient at answering the acute evolutionary question of success and spread.

This issue of distant versus closely-related species comparisons was enjoyably tossed around between myself and Antonio during a long coach trip to Niagara Falls in the late 1980's and under the canopy of water that kept us fully drenched as we were ferried close up to the falls. The scene must have seemed quite surreal to the other passengers who persistently asked us to keep our voices down and to try and make some attempt at appreciating the marvel surrounding us. But we were like dogs with a bone. My sense of place and occasion at Niagara Falls was defined more by wonderful memories of Antonio's vociferous arguments than by the thunder of the water which threatened to swallow us and our confounded biological obsessions.

Reflecting back on this discussion, it is clear that Antonio's approach to encourage wide species comparisons has borne fruit (for example, see diverse papers in Akam *et al.*, 1994) There is no

need, in this volume, to spell this out in detail. At the same time, the closely-related species approach (for which I give one example in Bonneton *et al.*, 1997) is beginning to take shape in several genera, and the sorts of questions answerable by such comparisons are being appreciated. The more distant future will inevitably need to probe further down into the within-species level of diversity, if we are to sharpen our evolutionary understanding.

What is at stake, in my view, (and I believe in that of Antonio's) is the molecular characterization of 'tolerance'; that is, the functional flexibility in the ways cells and organisms go about their business which permits evolutionary change to occur. The Darwinian view on this, is that 'tolerance' is a property of the external biotic and physical world. Toleration is a relative measure of reproductive success: mutation proposes, selection disposes. The strong message that I hear from Antonio, and with which I fully concur, is that tolerance is an internal matter. If consenting, functionally interactive, molecules and modules have a means of tolerating genetic novelty during ontogeny then novelty can be absorbed. The question is, then, where does this tolerance reside? This is not a metaphysical problem but a hard-nosed question concerning the molecular basis of the very 'generative and transformational rules' that are so widely shared across Kingdoms. It is my belief that tolerance of change and the requirement for the maintenance of internal consistency of essential cellular functions during a period of change, emerges from the high levels of genetic and functional redundancy in living organisms, coupled to the particular population dynamics of a variety of processes of genomic turnover. These widely documented phenomena (for reviews of literature see Dover 1992, 1993) provide the appropriate conditions for molecular coevolution to occur between interactive parts. In other words, they provide selection with the opportunity and the time to promote compensatory molecular changes in one partner molecule that help retain, or permit modifications to, essential functions, in response to mutational changes, driven by turnover, in the other partner molecule. Tolerance (or permissiveness) is an emergent property of redundant, modular systems that are subject to genomic mechanisms of turnover.

Hence, molecular interactions are not locked one into another, in some singular lock-and-key relationship honed by external selection; rather there is a looser and more relaxed relationship between redundant, interactive modules, participating in a variety of pleiotropic functions. In the same way, the population dynamics of genomic turnover (molecular drive) mean that there is a more relaxed relationship between organisms and their supposed external niches. Hence, the new developmental biology and the new genome biology dispense with the narrow view of locks-and-keys, both at the level of interacting molecules and at the level of organisms and their ecology. This is not to say that selection does not play a crucial role: indeed, molecular coevolution is not possible without the interplay between genomic turnover and selection. Nevertheless, selection is as busy with the events driven from within as it is busy with events driven by the ecology.

If there is one shining intellectual message that I've learned from Antonio it is the necessity to go deep inside organisms; for only through the molecular dissection of the transformational "rules" of development and through the experimental diachronic approach to evolving genomes can we begin to add anything useful to the work initiated by Darwin, over 150 years ago.

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