1

Drosophila melanogaster and the Development of Biology in the 20th Century

Alfonso Martinez Arias

> "When I see *Drosophila* under moderate magnification of a binocular microscope I marvel at the clearest form of the head with giant red eyes, the antennae, and elaborate mouth parts; at the arch of the sturdy thorax bearing a pair of beautifully iridescent, transparent wings and three pairs of legs……"
>
> C. Stern (1954) "Two or three bristles" *Am. Sci.* **42,** 213–247.

Summary

The fruit fly *Drosophila* has played a central role in the development of *biology* during the 20th century. First chosen as a convenient organism to test evolutionary theories soon became the central element in an elaborate, fruitful, and insightful research program dealing with the nature and function of the gene. Through the activities of TH Morgan and his students, *Drosophila* did more than any other organism to lay down the foundations of *genetics* as a discipline and a tool for *biology*. In the last third of the century, a judicious blend of classical genetics and molecular biology focused on some mutants affecting the pattern of the *Drosophila* larva and the adult, and unlocked the molecular mechanisms of development. Surprisingly, many of the genes identified in this exercise turned to be conserved across organisms. This observation provided a vista of universality at a fundamental level of biological activity. At the dawn of the 21st century, *Drosophila* continues to be center stage in the development of *biology* and to open new ways of seeing cells and to understand the construction and the functioning of organisms.

Key Words: Development; *Drosophila;* fruit fly; genes; genetics; history.

1. Introduction

Biology is about experiments rather than theories, about observation and description rather than prediction. For this reason, it is often difficult to separate what one knows from how one knows it, the observation from the method.

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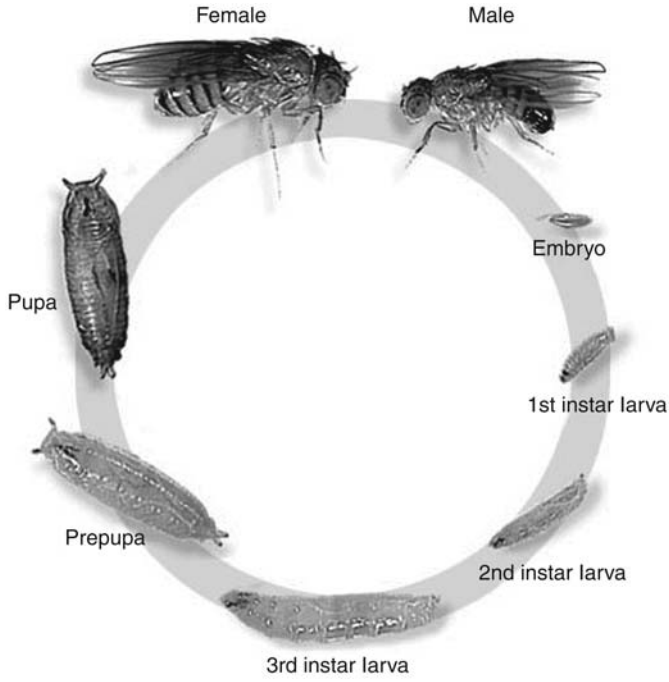

Fig. 1. Male and female *Drosophila* and their life cycle (courtesy of Christian Klämbt in FlyMove: http://flymove.uni-muenster.de/Homepage.html).

Once we notice the presence of wings in an organism we can check whether they are present in others; however, even if we see them in many, this does not allow us to say that they will be present in all: all birds have wings but mammals do not. The same is true at whichever level of organization one looks at. Successful predictions are rare in *biology*. It is perhaps, for this reason, that the notion of *model organism* has been crucial in the development of *biology* as a *science* in the 20th century. A model organism is one which allows us to analyze a particular problem in the hope that the answer it gives us will be general and perhaps universal. Thus, peas and plants in general, were essential to develop the key notions of *heredity*, finches for *evolution*, and bacteria for unravelling the molecular nature of the gene, the genetic code, and the fabric of metabolism. With few exceptions model organisms come and go and are limited to specific fields and moments but one has had a constant presence in the 20th century and has made significant contributions to multiple areas of *biology*: the fruit fly *Drosophila melanogaster*.

Drosophila **(Fig. 1)** was introduced as an experimental animal at the beginning of the 20th century, probably around 1901 in the context of evolutionary biology but soon became a workhorse of biological research ***(1,2)***. Its main

attributes were then the same as they are now: rapid generation time, ease and robustness of culturing, and low maintenance cost. Over the years it has left its mark in a wide range of questions from the nature and organization of the hereditary material to the effect of space trips on embryogenesis. However, much of this mark is not only by way of the concepts it has generated but also in terms of ways to approach problems that have been exported to other organisms. This volume is a compilation of methods used with *Drosophila* in the last few years to tackle a number of problems of cell biology and developmental genetics. The methods are described by active practitioners and therefore, have the flavor that only a cook can give to a meal. It is a very up-to-date compilation and adds significantly to others of a more general nature ***(3–5)***.

In this introductory chapter I have been asked to comment on *Drosophila* "as a model system." Instead of providing an annotated foreword to the technical chapters that follow, I have decided to illustrate the development of *Drosophila* as the sophisticated experimental organism that it is today and how this development has resulted from its rising to the challenge of specific biological problems. My account will be couched in a historical framework but this is no abridged history of the contributions of *Drosophila* to biological knowledge, a subject that would take more space than is available here and that probably would take a different approach.

2. TH Morgan, his Students, and the Foundations of Genetics

Around 1908, in room 613 of Schmerhorn Hall at Columbia University in New York, Thomas H. Morgan, an embryologist of some renown at the time, begins to grow *Drosophila* in large quantities with an interest in exploring the existence of what today we would call "macromutations." At the time, classical animal and plant breeding are turning into the new *science* of *genetics* and for people with an interest in the relationship between genes and phenotypes, mutations provide an intriguing, albeit mysterious, link. Sometime in 1910, Morgan came across a fly with white eyes, which was going to sidetrack him from his interests for 20 yr. This was the first allele of *white*, and its linkage to the sex chromosome ***(6)*** triggered a revolution in our understanding of heredity and led to the establishment of *genetics* as a subject with defined concepts and experimental methods.

Much has been made of how TH Morgan and his students Sturtevant, Bridges, and Muller laid down the foundations of modern *genetics* and I shall not dwell on this here (but *see* **refs.** ***1,2,7–9***). When Morgan begins to grow *Drosophila* in 1908, genes are hereditary particles in the abstract mendelian sense, a concept Morgan was suspicious of ***(1,6)***, without a subcellular location, fabric, or clear connection with their products, the phenotypes. By 1928 when Morgan moves to the California Institute of Technology to establish the biology division, there is an impressive edifice on which much of modern *biology* will be built.

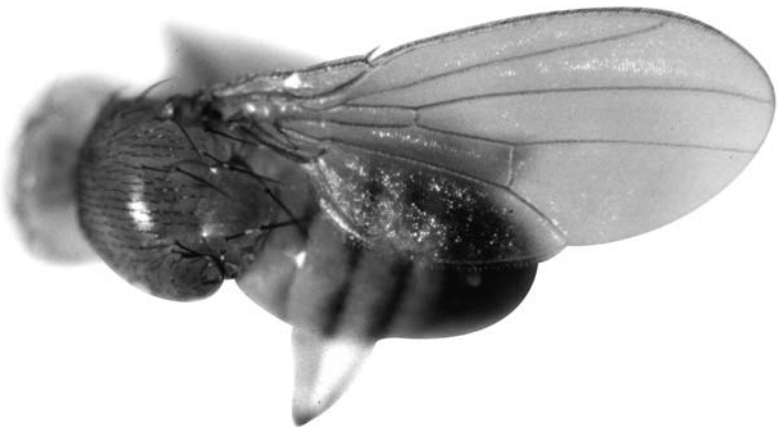

Fig. 2. Adult fly homozygous for the *wingless*[1] mutation, which leads to the loss of wing tissue and its transformation into notal tissue. Often the phenotype is not fully penetrant and only shows on one side, which by comparison with the other side, highlights its features (courtesy of Elizabeth Wilder).

In a period of about 20 yr, Morgan and his students showed the universality of Mendel's factors, that they could be arranged in linear order and that this order can be used to create genetic maps, that these factors exist in different forms, alleles, which can mutate in forward and reverse manner, and that their functioning depends on their position within the chromosome. In addition they demonstrated that the genes lie on the chromosomes and elucidated the existence of chromosomal aberrations (inversions, duplications, and deletions) from genetic data before observing them directly, i.e., they could use genetics as a predictive algebra for chromosomal structure. Altogether these findings created a foundation and a language, which enabled *biology* to progress for much of the 20th century. Finally, one of Morgan's students, H. Muller demonstrated that mutations could be induced by X-rays. In doing this he opened the door not only for the understanding of the chemical nature of the gene, but also, and perhaps most importantly, for the generation of alleles and mutants that have been an essential element of progress. At the same time with a system of balanced lethals, or what we call today "balancer" chromosomes, Muller introduced the ability to keep stable stocks, a system which is the envy of other organisms and which has enabled a huge amount of genetics in *Drosophila*.

In addition to concepts, a most important legacy of this period is mutants **(Fig. 2)**, carefully kept in stocks, some of which have survived almost 100 yr. They were used to probe into the nature of the gene and thus, mutations like *achaete* and *scute*, *bithorax*, or *Notch* served as battle grounds to define notions

like allele, dominance or recessiveness, cross over, *cis–trans* tests, and the logic of complex complementation or of gain and loss of function. However many of these genes were hiding other revelations for which the ground work of these years would prove invaluable. Inadvertently, *Drosophila* was doing the first homework of any model organism: good genetics and mutants.

3. Difficult Problems as Tractable Questions

TH Morgan was not a geneticist at heart and never stopped being an embryologist/developmental biologist. Two anecdotes betray this fact. Intriguingly, he only published one paper in the journal Genetics and this was on the subject of the obituary of C. Bridges (referred in **ref. *10***). In addition, and perhaps most significantly, after he moved to Caltech in 1928, he left Genetics to C. Bridges, A. Sturtevant, and his new recruits and returned to the embryological investigations with marine organisms that had occupied him in his pre-Columbia days ***(6,11)***. It some times appears as if, for Morgan, *genetics* was a necessary distraction, a "deviation" as S. Brenner has put it, to develop tools to tackle, with more hope of success, the issues that preoccupied him: the secrets of animal development and evolution. At several times in his career he tried to bridge the two fields ***(12,13)*** but never made much progress; although he did feel that *genetics* one day would provide the answers he was seeking ***(13)***. Notwithstanding this impasse, progress was being made in linking genes and embryos, although it took time to appreciate this.

Part of the difficulty to bridge the two subjects was that embryology is about embryos, and the Morgan school, engaged as it was in the abstractions that genes were at the time, never paid too much attention to what was going on inside fertilized eggs. In the late 1920s and 1930s, the embryogenesis of *Drosophila* began to be described ***(2)***. D. Poulson ***(14)*** did the most thorough work summarized in a classic article, which served as an obligatory reference until the publication of the treaty of J. A. Campos Ortega and V. Hartenstein ***(15)***, the standard modern reference, which describes the process with modern histological techniques **(Fig. 3)**.

Poulson did not simply describe the histology and development of the embryo, but also tread into the relationship between the genome and development. At the time it was felt that there was a correlation between the amount of chromosomal material and the development of the animal. This, no doubt, was derived from the classical experiments on dispermic fertilization of sea urchin eggs by T. Boveri, who showed a correlation between the number and identity of chromosomes present in an embryo and its degree of development ***(16)***. A quantitative correlation between chromosomes and development was reinforced by observations on the development of flies with large intrachromosomal deletions until the issue was systematically analyzed by Poulson in a study of the

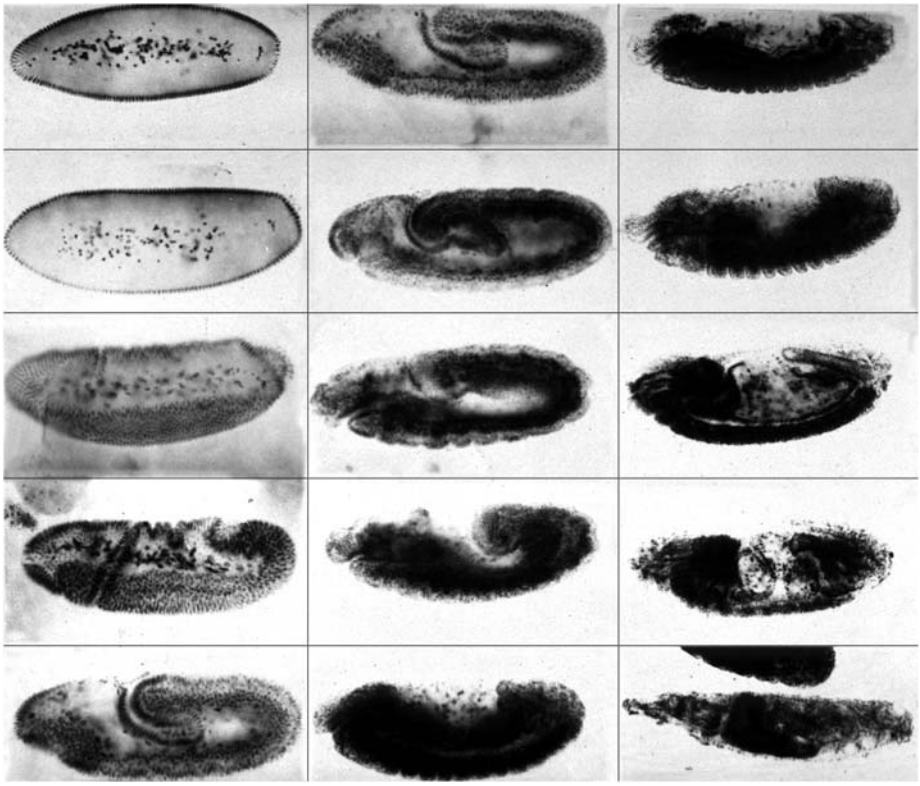

Fig. 3. Whole mounts of *Drosophila* embryos stained with Fuchsin (which highlights the nuclei) and which reveal the development and morphogenesis of different tissues. Blastoderm is at the top left and the hatching larva at the bottom right. The density in the stain is associated with an increase and density of cells. These preparations were very useful in the analysis of internal organs in the 1980s.

Notch region. Focusing on the phenotype of embryos mutant for *Notch*, which had precise defects in the development of the epidermis, the nervous system, and the mesoderm, he noted that the defects of embryos with variable deletions that included the *Notch* locus were the same as those bearing mutations in *Notch*, as long as the deletion included *Notch* ***(17)***, i.e., he established the first correlation between a gene and a specific developmental defect.

A few years later, in Switzerland, E. Hadorn coming from a tradition of experimental embryology began to use imaginal disks to ask similar and related questions. In the course of this work he developed techniques to generate mosaics between cells of different genotypes, which allowed him to probe into issues of cell differentiation and cell determination that were going to play an important role later on. Two important contributions of his work are the notion of stability

of certain differentiated states ***(18)*** and his treaty "developmental genetics and lethal factors" ***(19)***, which represents an important account and discussion of the significance of lethal mutations in a large number of organisms.

These studies were exceptions, and for the most part, work with *Drosophila* still focused on visible mutations in the adult, for example the effects on bristles of mutations in *achaete* and *scute*, many that affected the pattern of the wing and to a fewer extent, the legs. These mutations were used to probe the nature and behavior of the gene, but the connection between these mutations and the developmental events they affected took time to come to the fore. A particularly interesting and significant case in point is the development of the work of E. Lewis on the *bithorax* mutants, a collection of mutations initially uncovered by C. Bridges and which appeared to transform one segment of the fly into another. E. Lewis ***(20)*** began to study *bithorax* mutants to explore abstract notions of the fine structure of genes, but he slowly realized that they reflect homeotic transformations ***(21)*** and contain information about the relationship between genes and development ***(22,23)***. His work moved slowly from gene structure to gene function and pioneered a logic and an approach that were to prove very important for the developments of the 1980s ***(24)***.

After the 1930s, *Drosophila* moves out of the limelight, which becomes occupied by phages and *Escherichia coli*. The objective was to unravel the molecular nature of the gene and *Drosophila* was not the organism of choice for this task. However, *Drosophila* researchers continued to probe into other problems in the way they knew best: using mutations and the cook book of *genetics*. In doing this, it became steadily clear that *genetics* harnesses an enormous potential to turn difficult problems into a set of tractable and answerable questions, and much of the progress that led to the watershed of the end of the century relied on the steady accumulation of information and reagents that took place during the 1940s and the 1950s. It is along these lines that the work of E. Lewis tranforms itself from abstract genetics to the relationship between genes and development, and that of E. Hadorn from experimental embryology to developmental genetics and to cite another important example a few years later S. Benzer decides to use the fly to tackle problems of neurobiology and behavior with excellent dividends ***(25)***.

4. Mosaics

The significance of the work of Lewis and Hadorn would take some time to be noticed. There were several reasons for this and one of them is that in the context of developmental biology mutations can be dangerous weapons. It is good thing to have a mutation that disrupts a process and generates an abnormality but it is a different one to know the cause of the phenotype, as it is often not clear whether the phenotype is a direct or an indirect consequence of the mutation

(19). It is important to know if the effect that one observes is specific or not, whether it is instructive or responsive. Some of these issues were resolved by the introduction of mosaic analysis for the study of gene function, an approach that has now become widespread using the guidelines derived from *Drosophila*.

Development is not just about generating different tissues or structures but about organizing cells in space. It was C. Stern who first noticed that one could use genetics as an analytical tool to probe this problem and find the relationship between gene function and pattern ***(26)***. In his initial studies he used mutants in the *achaete/scute* complex, which affect the pattern of bristles in the adult. Seizing an observation by A. Sturtevant that it was possible to generate individual mosaic for particular mutations ***(27)*** he generated flies in which some of the epidermis was wild-type and some mutant for specific alleles of *achaete* and *scute*. The technique was a genetic enhancement of the loss of the X chromosome in females, which allowed the generation of gynandromorphs in which some of the tissue is male (XO) and some female (XX). If the fly contained a mutation in one of the X chromosomes, its phenotype reveals itself in the male tissue. Taking advantage that *achaete* and *scute* are on the X chromosome he analyzed their requirement in the generation of the pattern of bristles. He observed that bristles were affected only when the producing cells were mutant for *achaete* or *scute*, i.e., the mutations behaved cell autonomously ***(26)***. He extended these studies with mosaics obtained by mitotic recombination and concluded that these genes were involved in the read out or response to some underlying prepattern that he struggled to find for most of his scientific career ***(28)***. Stern's analysis of the *achaete/scute* complex has withstood well the passage of time and his conclusions served as an inspiration for further work on the nature and function of these genes ***(5,29,30)***. E. Lewis used the mosaic technique to analyze the mutations of the Bithorax complex (BX-C) and show that these genes, also, act in a cell autonomous manner ***(31)***. This work was the beginning of an important link between genes and cells.

The use of mosaics to analyze gene function was taken to a modern level of possibilities with the work of A. Garcia Bellido and his students. Garcia Bellido had worked with Hadorn and was aware of the possibilities of mosaics to analyze issues of determination and differentiation in imaginal disks. In the late 1960s, he went to Caltech to work with E. Lewis and to learn some of the genetic techniques that were being developed to analyze developmental events. His interactions with Sturtevant and Lewis led him to use genetic mosaics for the study of cell lineages and in collaboration with J. Merriam extended Stern's technique of mitotic recombination to generate large numbers of temporally controlled mosaics ***(32)***. This allowed him to trace precise lineages of different tissues and to ask very defined questions about gene requirements in time and space, observations that would lead him to the discovery of compartments ***(33)***

and to the connection between gene activity in the cellular realm with a particular emphasis on *bithorax* and the adult lineages ***(34)***.

The visit of A. Garcia Bellido to E. Lewis blent traditions and approaches in a manner that increased the conceptual and technical repertoire of *Drosophila* as a model organism. At about the same time, a parallel blending exercise was taking place at Yale when another student of Hadorn, W. Gehring, met the last student of Poulson, E. Wieschaus. While Garcia Bellido, Merriam, and Lewis were interested in adult lineages, gene function, and determination, Wieschaus and Gehring began to explore related issues in the embryo and its relationship to the imaginal disks ***(35)***. This fusion of Europe and the US was going to have important consequences and allow *Drosophila* to tackle as an important model system the problem of development.

Mosaic analysis was not just used to tackle simple developmental problems but began to have an important role in neurobiology where the pioneer work of Hotta and Benzer ***(36)*** attempted to map behaviors to particular loci and organs. Mosaic analysis is an indispensable tool of modern fly genetics and was taken to a level of ease and interest with the FLP system ***(37,38)*** and its extension to internal organs with the availability of histological markers ***(39,40)***. It is easy to underestimate the significance of these developments as mosaic analysis is, today, a tool of choice for the analysis of any gene within a developmental context with a significant impact in the analysis of vertebrate development.

5. The Molecular Nature of Genes

Morgan never gave up wondering about the links between *genetics* and *embryology* and in his Nobel address, considering how genes might regulate development remarked that "it is conceivable that different batteries of genes come into action one after another, as the embryo passes through its stages of development" ***(13)***. To test and probe into this remarkable statement required understanding the molecular nature and biology of the gene and the ability to identify, read, and interpret those batteries of genes. Unfortunately *Drosophila* was not the organism of choice to answer these questions. Solving these problems required the development of molecular genetics and molecular biology in bacteria and phage, which provided a new set of tools and concepts that made classical genetics more powerful.

Once the link between genes, DNA, and the genetic code became clear, and the technology to obtain and characterize DNA was at hand, one could turn to issues of how genes look like and what they encode. Answers to the genetic control of development or behavior were at hand. Waiting on the wings were *bithorax* and *achaete–scute*, *Antennapedia*, and *Notch*, and also genes involved in behavior like *Shaker* or circadian rythms like *period*. What did they encode? Enzymes? Structural proteins? A new kind of entity dedicated to development

and behavior? Anecdotes of those musings could fill many pages. If genetics and experimental embryology began to give us an inkling of the relationship between genes and cells, a second fusion exercise between molecular biology and developmental genetics broke the code of development and began to place names to the cogs and wheels of behavior.

6. Fusion Biology

The molecular era of *Drosophila* genetics begins with D. Hogness proposal in 1971 to use large overlapping pieces of DNA to obtain maps of chromosomes at a molecular resolution (referred in **ref. *41***). For the next few years this work leads to the development of techniques and materials, which allow the assembly and mapping of large chromosomal regions. Throughout the late 1970s, a collaboration between Hogness and Lewis initiates an assault on the BX-C through a fusion of molecular biology and classical genetics, spearheaded by W. Bender and P. Spierer ***(42)***. The BX-C is what the studies of E. Lewis had turned the collection of *bithorax* mutants into and which he interpreted as a gene complex regulating the differentiation of the posterior half of the fly ***(23)***. The objective of the collaboration was to obtain a molecular map of the region and uncover the functions of the resident genes. Shortly afterward two other foci of activity target a group of genes, the Antennapedia complex, identified by T. Kaufman and then hypothetically related to the BX-C. MP Scott in Bloomington and R. Garber with W. Gehring in Basel (Switzerland) undertook the exploration of the corresponding region of DNA. It might seem strange that hour long seminars on restriction maps and locations of inversions, insertions, and deletions filled rooms with excitement, but this was the way it was in the 1980s when everybody was reading much in those maps where often transcripts were not easy to spot. This work revealed an intriguing landscape of large transcription units with small exons and mutations peppering noncoding regions **(Fig. 4)** ***(42–45)***. The correlation with function required a visualization of the spatial distribution of the transcripts and it was M. Akam in the Hogness group who began to work out the methods to do this in *Drosophila* embryos and imaginal disks. The patterns of transcription that emerged were roughly in agreement with the requirements laid down by the genetics, i.e., genes were expressed in defined patterns that overlapped the regions in which they were required, but they also revealed surprising temporal dynamicity and intriguing tissue and cell type specificity **(Fig. 5)** ***(46,47)***. The mutants were the landmarks that colored the otherwise arid landscape of restriction maps of large DNA stretches and a picture of these complexes began to emerge. Much is rightly made of these specific studies, but it is important to mention how parallel studies were undertaken to analyze the molecular nature of genes shown to influence the development and activity of the nervous system, in particular *Notch* and *period*.

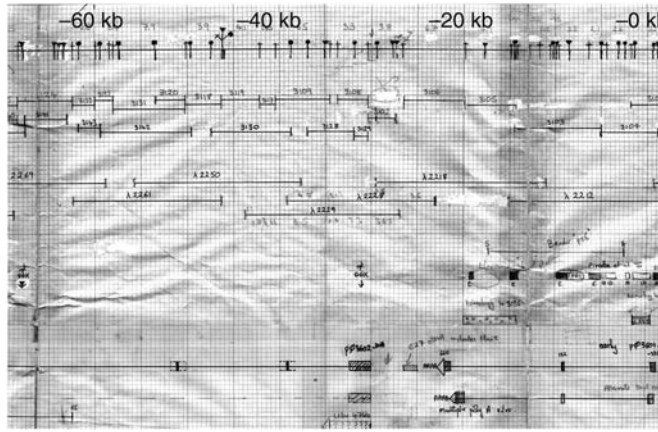

Fig. 4. Molecular map of the *Ultrabithorax* region of the BX-C as used by Michael Akam (modified from W. Bender) in his studies of the spatial expression of the *Ultrabithorax* gene (courtesy of Michael Akam).

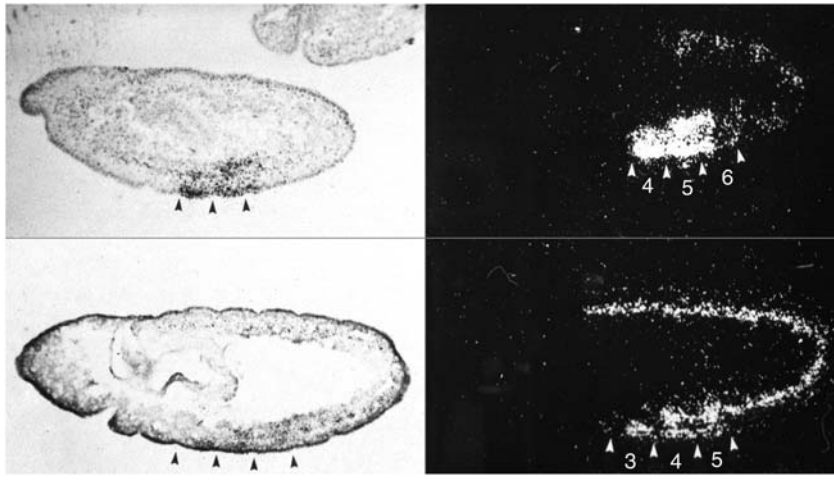

Fig. 5. Differential activity of the two promoters of the *Antennapedia* gene from the Antennapedia complex, as shown by radioactive (^{3}H) *in situ* hybridization to sections of stage 9 embryos. The P1 promoter is predominantly expressed in the ectoderm of parasegments (PS) 4 and 5 and the mesoderm, of PS5, which span the first and second thoracic segments, whereas the P2 promoter is expressed in the whole of the central nervous system and in the ectoderm of parasegments 3, 4, and 5. The frames on the left represent bright field images and those on the left dark field (Alfonso Martinez Arias).

The ability to manipulate and put genes back into organisms had been an important element in the success of *E. coli* and *Saccharomyces cerevisiae* to understand the molecular underpinnings of gene structure and function. This was achieved in *Drosophila* by an ingenious domestication of wild transposable elements, P elements, which were discovered in studies of wild-type *Drosophila* populations in the 1970s, as mutagenic elements. A. Spradling and G. Rubin generated a series of vectors and associated technology that allowed, albeit without control of location, the transformation into the genome of engineered pieces of *Drosophila* DNA ***(48)***. *Drosophila* was the first higher eukaryote to be transformed and this served as an inspiration for attempts in other organisms. The technique has continued to evolve and recent developments allow easy targeted insertion into the genome.

The molecular analysis of *Drosophila* genes was initiated by a fusion between classical genetics and molecular biology and the results proved the benefits of such exercise with high dividends, which would have an impact on the way similar problems would be tackled in other organisms.

7. Mutants, Stripes, and Boxes

At the beginning of the 1980s, methods had been developed to target any gene, to find out its sequence, analyze its regulatory logic, and observe its pattern of expression. It was low throughput and low resolution but it was good enough to begin to uncover the logic of development. The BX and ANT complexes had led the way and their revelations raised many questions: What did the gene products do? How was the tight spatial expression of these genes regulated? How did the patterns emerge? What had made these complexes such objects of interests was their *genetics*, and it was *genetics* again, which was going to lead to the answers to many of these questions with most unexpected results.

Mutants are the bread and butter of *Drosophila* biology and they had been collected in a haphazard manner through the years, sometimes as curiosities, others as ways of asking questions about chromosomal mechanics or gene structure. However, with the exception of the *period* gene and a few exceptions including a focus in the absence of disks ***(49)*** or sterility ***(50)***, screens for mutants affecting a particular function or structure had not been performed in a systematic way and most certainly not with embryonic development as an object. It was the realization of the meaning of the phenotypes of mutants like *Notch*, *Kruppel*, or *bicaudal* and particularly the observation by E. Lewis in his 1978 paper that the cuticle secreted by the embryo could be used as a readout of genetic processes ***(23)*** what changed things (**Fig. 6**). The potential of these observations was brought to bear by the systematic screens for lethals conducted by Nüsslein-Volhard and Wieschaus in collaboration with Jürgens at EMBL in Heidelberg ***(51–54)***. The mutant collection that resulted from this

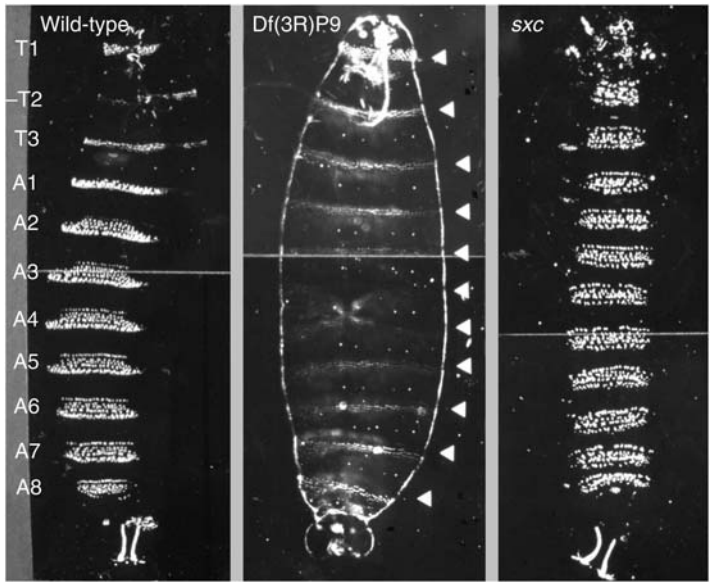

Fig. 6. Cuticles of embryos showing that the patterns of denticles are a readout of gene activity. Wild-type showing the thoracic (T) and abdominal (A) segments. *Df (3R) P9*, which removes the complete BX-C and results in all segments posterior to T2 developing like T2. *Sxc* mutant embryos in which all segments develop like A8 (courtesy of Phil Ingham).

effort uncovered a treasure trove, which provided the raw material for the molecular analysis of the corresponding genes. The emphasis turned from the adult to the embryo and what had been invisible until then revealed an intriguing choreography of stripes and spots. Disturbances in the pattern of the cuticle turned out to provide information about signaling, transcription, the cell cycle, the cytoskeleton, and cell adhesion. As a result of these findings, by the end of the 1980s the genetic regulatory network that establishes the coordinates and patterns of the early *Drosophila* embryo had been outlined ***(55)*** and with this work many now current notions about gene regulation in space and time were laid out. The notion of prepattern put forwarded by Stern ***(26,28)*** acquired a molecular ground in the observation that a gene expression pattern acts as a scaffold for a more complex one.

The cloning experiments of J. Gurdon in the 1960s had indicated that development was about controlling the expression of the genome in space and time ***(56)*** and *Drosophila* provided sound evidence for this. Furthermore, from very early on it became clear that development was about transcription and many of the mutants with pattern defects were mapped to genes encoding transcription factors. Also, there were some surprises and one of them was that these transcription

factors could be clustered into classes based on the structure of their DNA-binding domains. One of these domains, or boxes as they often were called, shared by members of the BX and ANT complexes ***(57,58)***, was termed the homeobox and seeded a large group of proteins with variations in the structure of this domain. The homeobox of the BX and ANT complexes turned out to be conserved in metazoa ***(59)*** and this structural conservation was accompanied with a functional one that provided the first glimpses of universality in the genetic make up of the developmental tool kit. The significance of these findings, with its roots in the genetic studies of E. Lewis, represent another highlight in the history of biology and vindicate *Drosophila* as a significant model system. It is possible that the homeobox would have been found by genome research methods, but it is only the work in *Drosophila* that provided the context within which to interpret what otherwise would have been a conserved protein-coding domain.

Soon, as the genes identified in the large Heidelberg screens began to be analyzed at the molecular level, a picture emerged of development being driven by transcriptional networks modulated by signaling molecules. The prescient comment of Morgan in his Nobel lecture became a reality: there were batteries of genes operating in spatio-temporal sequences. The molecular analysis of the existing mutants not only yielded a large number of new transcription factors but also new signaling pathways. Thus, to the then familiar EGF/FGF-Ras-MAPK were added other with the colorful names of Wnt and Hedgehog. The development of enhancer traps ***(60)*** and of techniques to establish spatio-temporal control of gene expression ***(61)***, which followed related techniques that had proven very successful in *E. coli* and yeast, added to an arsenal that developed as fast as it was making new findings about the structure and function of genes. As a result of this effort *Drosophila* was seen in a new light ***(61)***.

8. From More Genes to Genomes

The study of the genes that are required to make a fly embryo revealed that very few are dedicated to this task. It quickly became clear that genes are just tools that configure circuits and that most of those involved in early development are redeployed later in the imaginal disks ***(5,62)***. Slowly a picture emerged of how the very large number of cell types and tissue patterns required to make a fly resulted from combinatorials and redeployment of a few genes. The same picture of course is true of other organisms and, like the homeoboxes, the "fly genes" pop up in mice and humans as they do in jellyfish. The lesson was clear: what defines an organism is not its genes but their networks. Furthermore, the genes appeared to be conserved.

These conclusions were drawn from the work of many laboratories and individuals but the contribution of the laboratory of G. Rubin in Berkeley, California from the mid-1980s until 2000 is particularly significant. Using the

compound eye as an experimental system with a blend of classical and molecular genetics, mosaic analysis, and modern cell biology, several generations of postdocs and graduate students pursued the mechanisms required to specify and pattern the cells that make up the ommatidia of the fly. The result is a large harvest of genes, interactions, and mechanisms in the best Morgan tradition and one that has contributed very significantly to the modern development of *Drosophila* as a model system (*see* as two examples **refs.** ***63*** and ***64)***. With hindsight it is surprising that a structure so specialized has revealed so much that is universal; but the power might not lie in the structure, the bristle can be deemed to have done as much, but it was the method, above all, the genetics and the sustained and concentrated energy.

In the late 1990s, sequencing of genomes had become an important priority of the biological community. The main thrust was to decipher the information hidden in the human genome but, as ever, work on model organisms was paving the way for this enterprise. *Caenorhabditis elegans* was the first organism to display its genome ***(65)***, and *Drosophila*, with a more complex genome, its long tradition and high standing in the field of *genetics* was coming along at the pace and with the returns of other genome projects. It is at this time that C. Venter proposed a radical new sequencing approach to the human genome. In contrast to the ordered and progressive sequencing in vogue at the time he proposed shotgun cloning, large-scale sequencing, and computer programming power to assemble the genome. To test the method he looked for a suitable organism, and *Drosophila*, with a complex genome and a scaffold in place against which to test the sequence was an appealing prospect. And so, within a short period of time, the shotgun approach pioneered by Celera was shown to function ***(66,67)***. The publication in 2000 of the sequence of the euchromatic genome of *Drosophila* ***(67,68)*** and its analysis was a landmark in genome research and opened a bright new era of possibilities and information with which to take the information accumulated over the years.

9. Of Flies and Men

In 1794, William Blake dedicates one of his "Songs of innocence and of experience" to a fly and ends by musing: "am I not a fly like thee or art not thou a man like me?" Two hundred years later there might be reason to believe that there was something in this statement, for it is in the similarities in genetic make up and programming logic between flies and humans that *Drosophila* reveals itself as a consumate model organism ***(69–71)***. Fortunate as this might be, it is also true. Did we think that lungs would be made in such a similar fashion to the trachea of the fly ***(72)***? Did we think that the development of the fly nervous system was going to have the pervasive influence that it has on the development and function of the vertebrate nervous system ***(73,74)***? Did we think

that stem cells were going to find a model system in the fly ***(75)*** or that we could use the fly to study the cellular basis of cancer ***(76–78)***? Perhaps most significantly, over 70% of the proteins involved in the disease in humans exist in the fly and this means that the point can be made that *Drosophila* may act as a model system for human disease ***(69,70,79)***, learning and behavior ***(80)***, and even alcoholism and addiction ***(81)***.

The ability to shuttle at the genetic level between *Drosophila* and other organisms, and humans in particular, is founded on the conservation of the core genome. Whenever a gene is highlighted by its association with a disease, it is not surprising that the first port of enquiry is the multiple databases associated with the biology of *Drosophila* (*see* Chapter 3). If there is a similar gene (and chances are high that this will be the case), it is then easy to find useful information about its possible function, biochemical properties, and interactions and thus gain an entry for further studies. However, the main reason why this exercise is useful is not simply because of the conservation, which would have been highlighted by the genome projects and would have just left (as it does in many cases) a structural puzzle difficult to solve. The reason why the exercise is useful is because the deep and rigorous genetic analysis performed in *Drosophila* tells about function and molecular relationships of the gene product in a manner that, with the possible exception of *C. elegans*, is unparalleled. The true Rosetta stone of modern biology is not the homeobox ***(82)***, but the whole genome, and the Champolions are the many workers that gene-by-gene have laid a foundation on which much of modern *biology* is, like it or not, built on.

A short reflection on *C. elegans* might be in place here for it is clear that the worm has played a significant role in the modern era of *genetics*, particularly *developmental genetics*, side-by-side *Drosophila*, and it would not be fair to forget about this. However, for a number of reasons associated with its mode of development and possibly its evolutionary position, the fly has turned out to be a better model system for the vertebrates. This might be because the worm, as the highly specialized system that it is, does not have proliferation-dependent construction of organs and structures and has a very peculiar organization of its nervous system, which is not easy to relate to the operation of vertebrate systems. Be that as it may, despite these strategic issues, which play in favor of the fly when thinking about many aspects of human biology, it would not be right to forget the essential role that *C. elegans* has played in identifying many of the components of the molecular toolkit of an organism and establishing their universality.

10. *Drosophila* in the Postgenomic Era

There is little doubt that *Drosophila* has been an exceptionally useful model organism throughout the 20th century. Its most long-lasting legacy will perhaps be the practical demonstration that *genetics* is the language of *biology*. Much as

mathematics is the language of *physics*, *genetics* allows us to transform abstract problems into concrete experimental questions, which can then be answered through the application of established rules and operations. The formulation of mutant screens tailored to specific problems is, arguably, the best example of the power of this language ***(51–54,63,64,83)***. As a result of this endeavor, today *Drosophila* is not just a model organism but a reference for other organisms and most notably for humans. None of this will change as we move into the 21st century, but, is there more to come? What about the postgenomic/systems era? Will *Drosophila* be diluted in a sea of model organisms? Will it become a virtual reference more than an actual model for a *new biology*? How will *Drosophila* fare with the questions that will emerge in the near future?

We are in a time of transition. Where there were defined big questions that could be answered from many different points of view, today we have many deceptively small questions, all and each, probably interesting. Where we have had fusions of *genetics* and *experimental embryology* ***(34)***, and *genetics* and *molecular biology* ***(41)***, today we have *genetics* and *cell biology* converging to reveal intriguing aspects of the structure and function of the cell and to raise a wealth of questions, which we had not thought of before about protein function in cellular contexts ***(84)***. The ability to visualize molecules in space and time in living cells is one of the important drivers of this new perspective of cells and organisms, and *Drosophila* is playing an important role in the development of these techniques. But if something is changing more rapidly than anything else is the way we observe. Where before there were EMS, X-rays, and flies, today there are (in addition) *Drosophila* tissue culture cells and dsRNA, which allow rapid whole genome screens targeted to particular functions to be analyzed in cellular context (e.g., **refs.** ***85–87***). Instead of looking at one gene at a time, the microarray technology allows us to monitor the activity of the whole genome under defined conditions and it is the activity of the whole genome that becomes the phenotype we look at ***(88,89)***. All these developments are being used to delve into fundamental and general principles of the role, which ensembles of genes and cells play in the organization and function of *Drosophila* and by extension other organisms. Most remarkably, these observations are also having a big impact in *evolutionary biology* where through the extrapolation of these findings to other organisms, it has been possible to bring developmental biology into the fold of classical evolutionary theory and create the new discipline of "EvoDevo" deeply rooted in what we have learnt about *Drosophila* **(Fig. 7)** ***(90,91)***.

These developments will pave the way to the future, but the most interesting aspect of the future is that it is unknown, unpredictable and that although its seeds lie in the present we only see this with hindsight. In this regard, it is perhaps pertinent to ask about questions that remain to be answered and problems that we have not seen or that perhaps, in the deluge of genes of the last few

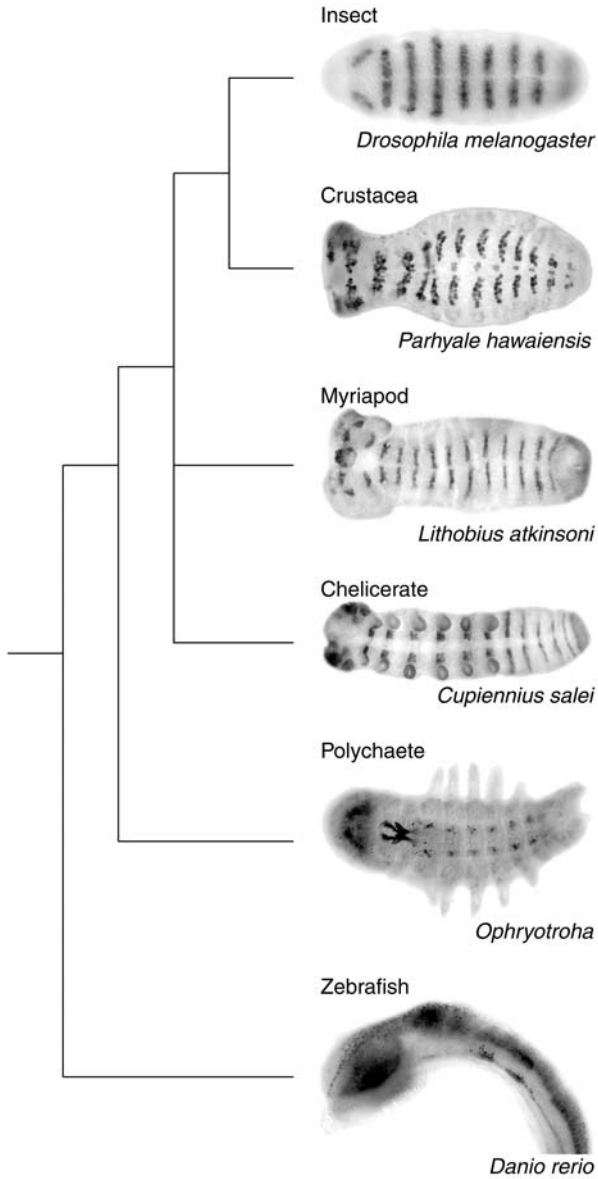

Fig. 7. Expression of *Pax3/7* family in embryos of various species revealed with Mabs DP311/DP312. In *Drosophila* this antibody recognizes the paired and gooseberry genes and, as in other arthropods, delineates a subset of neuroectodermal cells in each segment. In the annelid shown, the staining is mostly neural and in zebrafish it highlights a subset of cells in the neural tube and the neural crest. Notice how the pattern can be easily related from one organism to another indicating that not only the sequence of the gene and probably its function have been conserved but also the regulatory network associated with the regulation of its expression (courtesy of Nipam Patel and Greg Davis).

years, lie dormant or we have forgotten. Are there important biological questions left in need of an answer? Can we enunciate them and test them in our favorite model organism? Will *Drosophila* succumb to the temptation of becoming a genomic cottage industry at the service of an ever-increasing number of publications and databases? Or will it rise to new challenges and once more show its potential to find general solutions? What are or can be those questions? Of course, the most interesting one probably escape our attention but at the moment there are some themes emerging, which might play a role in the future. One of them, as hinted at previously, is the new *cell biology* driven by live imaging and the links it poses between structure and function. Most important though, biology is becoming quantitative because we can measure variables unthinkable a few years ago. In this exercise we realize that, at the molecular level, the processes we observe are subject to fluctuations that sometimes are used and sometimes are dampened to generate cell fates or pattern them in space. Appreciating this is leading us to analyze the mechanisms that regulate the transitions from stochastic molecular events to smooth and deterministic cellular processes **(Fig. 8)** ***(92–95)***. As a result of the precision in the analysis of some events, like the patterning of the blastoderm, *Drosophila* emerges as an important reference for modeling and *synthetic biology*, which aims at reproducing from first principles the circuits that produce stable patterns in space and time ***(96)***.

An important task in the immediate future is to unravel how the components uncovered in the 20th century are put together to make up a functional organism. It is almost certain that this will yield significant insights into biological processes and that we shall discover new laws in *biology*. In this enterprise, *Drosophila* is likely to emerge again as a useful model organism, as at the beginning of the 21st century it is still true that "When with foresight and luck Morgan selected this species for studies in heredity and together with Sturtevant, Bridges, and Muller derived from it the evidence for the existence, arrangement, and complex transmission of genes in the chromosomes, the significance of the results was not owing to *Drosophila* as a unique organism, but as a representative of all organisms" ***(27)***.

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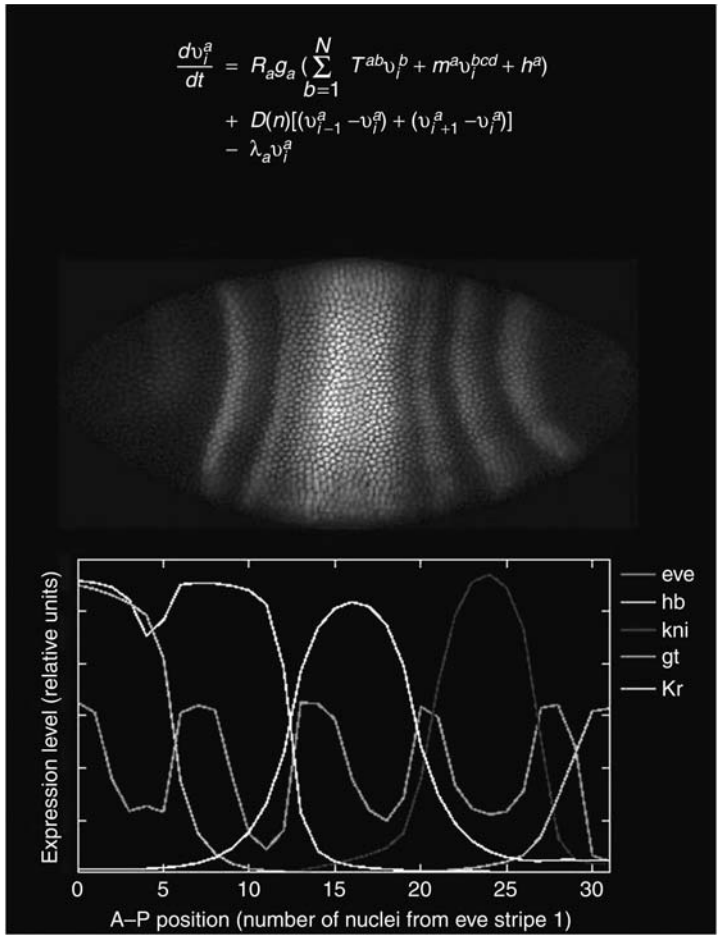

Fig. 8. A quantitative analysis of gene expression during the deployment of *gap* gene expression allows the development of models and simulations that can reveal the wiring and functioning of gene networks (courtesy of John Reinitz and *see* http://flyex.ams.sunysb.edu/).

perspective from which I look at the field (I do not think it is a bad one). I have nonetheless tried to point out here and there how *Drosophila* has and does contribute to the other big questions of *biology* like the development and function of the nervous system or evolutionary biology. If at times I have oversimplified or omitted significant contributions, it was not intentional. As I indicate at the outset, this is no history of *Drosophila* but a glimpse at the track record of *Drosophila* as model organism extraordinaire. The real history would require more space and, in this day and age, perhaps a different format. My work is funded by The Wellcome Trust and the BBSRC.

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