Developmental Biology 484 (2022) 12–21

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

An exciting period of Drosophila developmental biology: Of imaginal discs, clones, compartments, parasegments and homeotic genes"

Ginés Morata [a,*], Peter Lawrence [b]

[a] Centro de Biología Molecular, CSIC-UAM, Madrid, Spain
[b] Department of Zoology and MRC Laboratory of Molecular Biology, University of Cambridge, UK

ARTICLE INFO

Keywords:
Drosophila
Clonal analysis
Compartments
BX-C
Hox genes

ABSTRACT

In this review we recall a number of important discoveries that took place in Drosophila during seventies and eighties of the last century. The development of cell lineage methods and of powerful modifications of same, such as the Minute technique, led to the discovery of compartments and provided a clearer picture of the body organization: it came to be seen as a chain of metameric lineage units along the A/P body axis. Further, genetic screens allowed the identification of the genes involved in the establishment of the metameric scaffold — the segmentation genes— and also of Hox genes that are responsible for the specific development of individual body parts. As cloning methods became available, many of the most relevant of these developmental genes were cloned and a molecular analysis of development initiated. The discovery of the homeobox, a molecular mark of the Hox and other relevant developmental genes, allowed the finding of Hox genes in animal species, like humans, in which they could not be identified by genetic methods. Analysis of the structure and function of Hox genes provided a general image of the genetic design of the metazoan body.

1. Introduction

Here we tell what we remember of an exciting phase in the analysis of the developmental biology of *Drosophila*, covering roughly the seventies and eighties of last century. New techniques were exploited and from these results came ideas that spread out far beyond *Drosophila* itself. The universality of these hypotheses broke apart the viewpoint that "proper" embryology should be done on vertebrates and that insects were fundamentally different and therefore somewhat irrelevant. Even previously obscure aspects of entomology began to grab more general attention. At that time transplantation and genetic mosaics were being applied to study the building and design of the adult fly. The application of genetic and molecular knowledge to the analysis of developmental problems was relatively embryonic but had increasing influence. Although pure genetics was still handicapped by an obsession with inheritance per se, it had begun to break free and ask instead how genes act to build animals from simpler embryos. We focus this review on the imaginal discs, as they were at the center of this story, but it will become apparent that important work was also done in embryos. And, of course, there were many other notable researches and researchers in that period, but we have been asked to reflect on our own experiences.

2. Imaginal discs. Growth and transplantation experiments

Drosophila is a holometabolous insect, in which adult structures do not derive from larval ones but from groups of cells that are sequestered and grow separately during the larval period and then, during metamorphosis, differentiate the adult parts, piecemeal. Most of the adult cuticle derives from sac-like structures called imaginal discs; they do not have a function during the larval period and simply use the hemolymph of the larvae and its oxygen supply like a culture medium. The imaginal discs are named after the different cuticular structures they form during metamorphosis such as the wings, legs, eyes, antennae. They become individualized during the first larval stage as small groups of 20–60 cells (Madhavan and Schneiderman, 1977; Garcia-Bellido and Merriam, 1971; Martín et al., 2009), these cells grow during the entire larval period up to the onset of pupariation. The imaginal discs are a convenient experimental system to study development; they are easy to identify and isolate and can be subjected to many experimental and genetic manipulations.

A useful feature of imaginal discs is that they can be transplanted into the fluid-filled body cavity of adult females where they grow well (Beadle and Ephrussi, 1936). They can then be transplanted back into mature larvae and their metamorphosis induced. Transplantation experiments performed in Hadorn's laboratory (reviewed in Gehring, 1972)

* Corresponding author.
E-mail addresses: gmorata@cbm.csic.es (G. Morata), pal38@cam.ac.uk (P. Lawrence).

https://doi.org/10.1016/j.ydbio.2022.01.008
Received 30 September 2021; Received in revised form 17 January 2022; Accepted 18 January 2022
Available online 2 February 2022

established that the discs of mature larvae are already committed to differentiate a particular adult structure —even though their cells did not yet secrete any cuticle. The Swiss School drew on this distinction between commitment to form a structure ("the state of determination") and the actual "differentiation" itself. Since each disc had a characteristic morphology, the investigators could study the state of determination of cells located in different regions by cutting out a piece and inducing metamorphosis of just that piece. Work by Peter Bryant (1975) showed that at the end of the larval period mature wing discs have attained many states of determination; different regions are already committed to form the different parts of the adult structures. By the end of the larval period their program is finalized. Thus the discs provide us with an almost complete developmental sceneplay, from their identification in the embryo, followed by continuous growth in cell number to the late third stage when cells of many different commitments have become arranged into a complex pattern.

An interesting feature of imaginal disc cells is that their states of determination are stable; after serial transplantation into female hosts the individual disc cells can be induced to divide many times but generally maintain their original determination, for example wing disc cells keep differentiating wing cuticle with wing hairs, leg disc cells characteristic leg bristles and so on. But work from Hadorn's group (Hadorn, 1968) showed that imaginal cells can sometimes change their determined state from, say, wing to leg. This process was termed transdetermination. Transdetermination was found not to occur in isolated cells, but to be a collective action taken by a group of cells (Gehring, 1967). This posed a fascinating problem, not yet resolved, of how a group of cells might work together to take up a new (and the same) developmental status. There may be interactions between the cells that lead to this communal transformation or some decision might be imposed by some pervasive factor emanating from elsewhere; but the mechanism is not known. Collective decisions in development may not be uncommon; indeed, as we discuss below, the subdivisions into compartments are also taken by groups of cells.

3. Clonal analysis of imaginal discs

Clonal analysis is a key method in developmental studies. An individual cell is labelled with an indelible mark that can be passed on to the cell's progeny, which will form a clone consisting of the marked descendants of that original cell. Clonal analysis has been extensively used to investigate the development of the imaginal discs. In the early studies (Bryant and Schneiderman 1969; Bryant, 1970; Garcia-Bellido and Merriam, 1971) the only markers available were recessive mutations like *yellow, singed, forked, multiple wing hairs*, which alter the shape or the colour of the adult cuticular structures without altering their nature; they are "gratuitous" markers, which means that they mark the cells without altering in any way their behaviour during development (Lawrence et al., 1986). Use of these markers allowed the clones to be identified and studied in the adult cuticle. Clones were generated by XRay-induced mitotic recombination (MR) (Fig. 1a), which produced cells homozygous for the marker mutation; these transmitted that genotype to their progeny. The MR events occur during the G2 phase of the division cycle and the marked cell appears a few hours later at the G1 of the next cycle, thus the moment of initiation of the clone roughly coincides with the time of irradiation.

Clonal analysis provides information about the developmental potential of the original cell at the time it was labelled (for example if a single clone later gives rise to two cell types, then the potential of the primordial cell at the labelling event must have included at least these two states). From analysis of the clones one can deduce the number of cell divisions, the division rate as well the migratory and any mixing behaviour of the descendent cells, for example with immediate neighbours (Bryant, 1970; Garcia-Bellido and Merriam, 1971). These authors found that cell divisions occur during the larval period and that the overall division rate is approximately 9–10 h per division. They also estimated that the initial group of cells in the wing disc was about 50, in good agreement with direct counting (Madhavan and Schneiderman, 1977). One interesting observation made by Bryant (1970) and

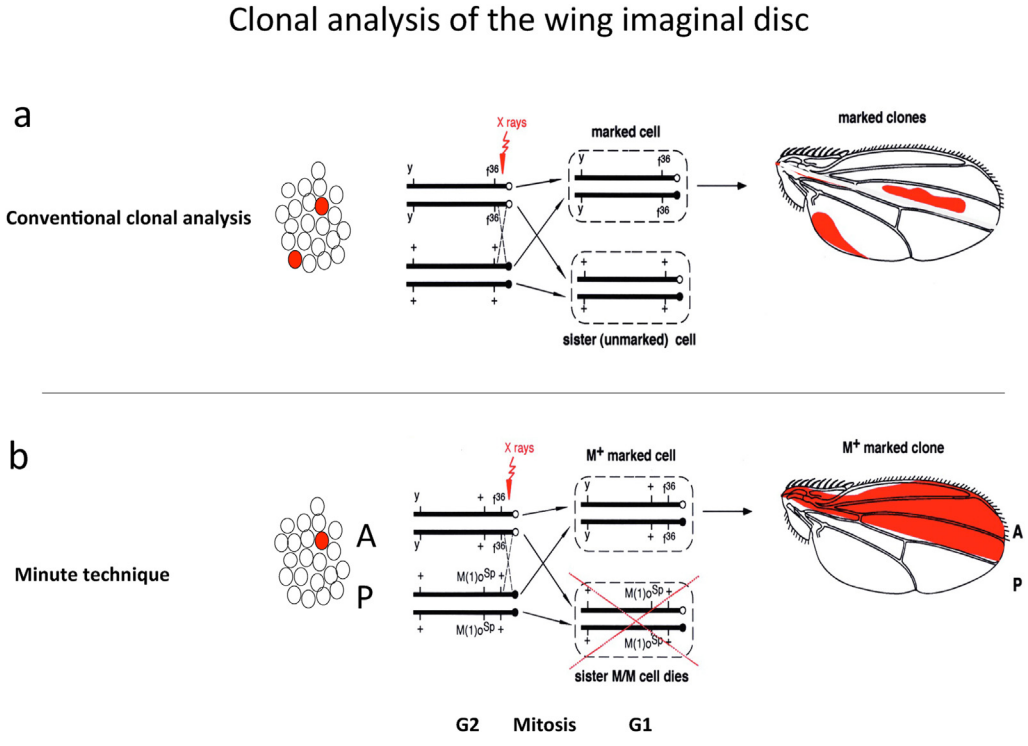

Fig. 1. Generating marked clones by X-Ray induced mitotic recombination (MR). a) Cells heterozygous for recessive markers like *yellow* and *forked*, which alter the colour or the shape of cuticular elements, are of normal phenotype, but after irradiation some cells (in red, in the scheme) in G2 period suffer MR that may result in homozygosis for these markers. The descendants of these homozygous cells will form a clone that can be recognized in the adult cuticle, as illustrated in the drawing of a wing to the right. b) The Minute technique is a variation of the method in a): it introduces a dominant *Minute* mutation ($M(1)o^{SP}$ in the figure) *in trans* with the marker mutants. After MR the marked cell (red) loses the retarding Minute condition and acquires a normal cell division rate, about 50–70% higher than surrounding $M(1)o^{SP}/+$ cells. The marked clones generated with this method can reach gigantic size, illustrated in the wing to the right.

Garcia-Bellido and Merriam (1971) was that when clones were generated after a specific time during the larval period their cellular descendants did not cross the boundary between the dorsal and ventral surfaces of the wing; consequently all their descendants were confined to the dorsal or ventral regions. It was a first indication of a developmental restriction in the discs.

At the same time and working with induced clones in another insect, the milkweed bug *Oncopeltus*, Peter Lawrence found lineage restrictions during the development of the abdominal epidermis (Lawrence, 1973). In this case, when a single cell was marked in an early stage embryo it produced descendants confined to within a segment and strikingly also to within either an anterior or posterior portion of each segment (Fig. 2). These clones thereby delineated segmental and intersegmental borders at fixed places in the anatomy. The sagacious insect embryologist Klaus Sander pointed out to Lawrence that these observations, showing that the segment was subdivided into anterior and posterior lineage domains, could also be related to the discovery of Lewis that mutations in the Bithorax complex, see later, also had limited realms of action within a segment. This proved to be an insightful thought.

4. The Minute technique and the discovery of compartments

The Minute technique (Morata and Ripoll, 1975) is essentially a modification of conventional clonal analysis (Fig. 1b). It is based on the usage of a class of dominant mutations, called *Minute* (Lindsley and Grell, 1968), that cause a delay in development. The Minute genes encode ribosomal proteins (Marygold et al., 2007), and having only one dose of a *Minute* allele reduces the normal level of protein synthesis, which presumably causes the delay. The key difference from conventional clonal analysis is that the marked clones have a proliferative advantage over neighbouring cells (Fig. 1b). While surrounding *Minute* cells divide at slow rate, the marked cells, which we refer to as M^+, divide at a wildtype rate, which, depending on which *Minute* allele is used, may be as much as 50–70% higher. As they grow in a slow-developing *Minute* larva, the M^+ clones have in effect an extra 48–72 h, sufficient to perform 6-8 additional divisions. Thus those clones can reach a very large size. In fact, considering the proliferative advantage, some M^+ clones induced early in development have the potential to make an entire wing.

The striking result found was that, in spite of their growth potential, the M^+ clones could not trespass over certain borders in the wing. The restriction that applied to the dorsal and ventral layers of the wing was not surprising because it has been suggested by previous work (Bryant, 1970; Garcia-Bellido and Merriam, 1971). But what was totally unanticipated was the existence of a lineage boundary running in the middle of the wing that subdivides it into two roughly equal regions, the anterior (A) and the posterior (P) compartments. It was remarkable that the A/P boundary is totally straight yet normally invisible, not being associated with any morphological feature visible in the cuticle. The cuticle made by cells at both sides of the A/P line looks identical, yet has a very different lineage.

This finding was as exciting as it was counterintuitive. What would be the function of a line in the middle of the wing separating seemingly identical cells? Incredulity was shown by the preeminent English physiologist Sir Vincent Wigglesworth at a Royal Society meeting in 1976 "I am sure Peter Lawrence is right and all this is fascinating, but what are these compartments for?" Seymour Benzer found the result very unexpected and wrote to Lawrence in 1976 asking for examples: Lawrence posted Benzer slides of wings bearing marked clones that respected the line. Benzer wrote: "The sharpness of the line separating anterior from posterior is really spectacular and its consistency of its position is quite impressive" But, as some others, he still had "lingering doubts" that the line might be caused by folding during development but he did not hold "strongly to this point of view, since it obviously failed to explain several other impressive phenomena." The folding theory, which was advocated by leaders such as Walter Gehring and called "the construction hypothesis" survived for years to become one of many monuments to those who think they know how animals should be built. Expectation can easily engender mistakes in Developmental Biology, which often contains surprises.

Then, using the Minute technique to analyze the lineage of different imaginal structures, it was shown that in addition to the wing disc, the subdivision into A and P compartments occurred in other imaginal discs such as the leg (Steiner, 1976), eye-antenna (Morata and Lawrence,

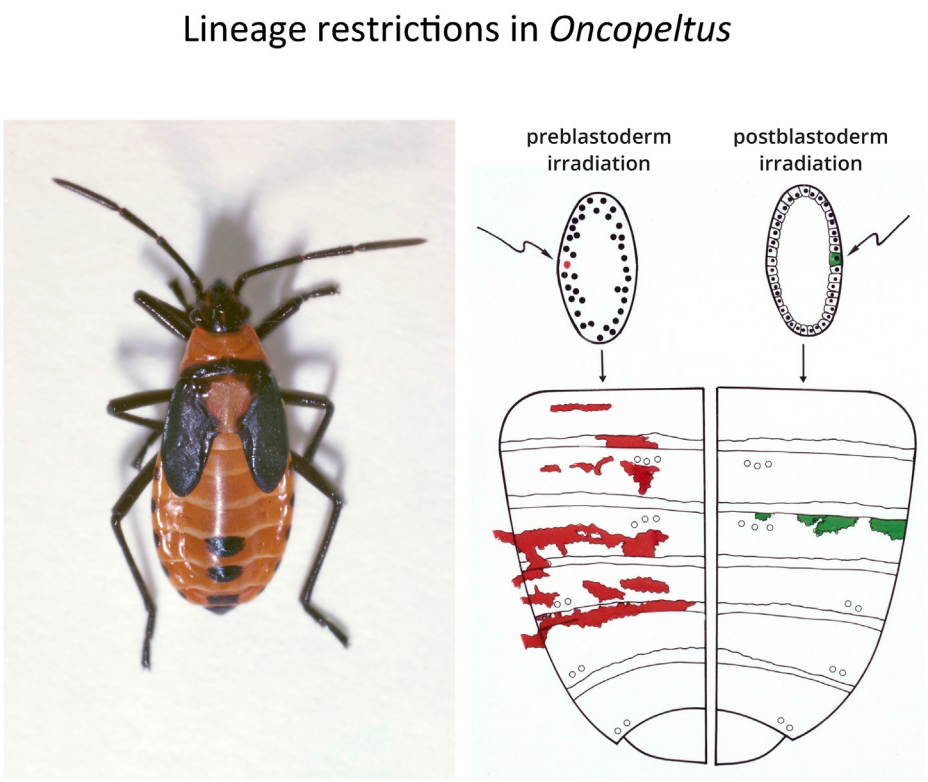

Fig. 2. The figure shows 5th instar larval *Oncopeltus fasciatus* and the consequences of irradiation before and after the blastoderm stage in the embryo. Before blastoderm the abdominal nuclei are not committed to contribute to a particular compartment and consequently clones induced by irradiation may have descendants in more than one segment, and/or compartment (a single clone consisting of thousands of cells is shown in red). Irradiation after blastoderm, when nuclei are committed to contributing to a particular compartment, produces clones with constituent cells restricted to one segment or compartment (a single clone consisting of hundreds of cells is shown in green) (After Lawrence, 1973).

1978), proboscis (Struhl, 1981) and in the abdominal histoblasts (Kornberg, 1981a).

Moreover, and importantly, the A/P boundary is established in early embryogenesis. In *Oncopeltus*, Lawrence (1973) (Fig. 2) and in *Drosophila*, Lawrence and Morata, 1977), before the imaginal discs are formed (Fig. 3). The latter result is of special interest in connection with a previous finding by Wieschaus and Gehring, (1976)). These authors had found that the progeny of clones generated at blastoderm or shortly after was restricted to within individual segments of the adult fly. The implication was that, although not visible by morphology, the early embryo is already subdivided into segmental primordia. The usage of the Minute technique by Lawrence and Morata, (1977) confirmed the findings of Wieschaus and Gehring, and in addition they showed that each primordial segment is already subdivided into A and P compartments in the early embryo. The P compartments are smaller, about 1/3 in cell number of the A compartments.

The early subdivision into compartments provided a novel image of the organization of the *Drosophila* body along the posterior axis: a chain of subunits: .P-A-P-A-P-A-P-A-P- (Fig. 3). It is a major feature of the body design, established in early embryogenesis and preserved for the rest of development. Note that this pattern can be arranged into metameric units, A-P or P-A, each containing an A and a smaller P compartment. The P-A subunit was defined as a parasegment and, as another counterintuitive surprise, appears to be to be defined earlier in development than the segment (Martinez-Arias and Lawrence, 1985), to be older in evolution and to be widespread in animals, even ranging as far as vertebrates (where the rhombomeres appear to be equivalent to parasegments (Fraser et al., 1990). Thus the metameric organization we observe in the body of *Drosophila* is a reflection of the original bauplan and therefore, the mechanisms that generate morphological diversity along the anteroposterior body axis must operate upon this scaffold.

One key feature of compartments is that the developmental decision to belong to a compartment is adopted together by a group of cells, a "polyclone" (Crick and Lawrence, 1975), a phenomenon whose mechanism has not been fully investigated but clearly depends on the position and neighbour relations of the cells. There are other examples of collective decisions; Gurdon (1988) described in *Xenopus* that the decision to develop as a muscle cell occurs in neighbouring cells differentiating in the same way at the same time. He called this a "community effect" a more descriptive than mechanistic term that might apply more generally in development to the formation of distinct blocks of tissue from sheets of cells. As we have seen transdetermination in *in vivo* culture in *Drosophila* is another example of such a collective decision. These processes may be commonplace but the underlying mechanisms remain mysterious.

5. Cell competition

Outshone by the discovery of compartments, the paper by Morata and Ripoll, (1975) contained another intriguing observation. When, instead of generating wildtype-growing clones in *Minute* larvae, they induced slow-growing *Minute* clones in wildtype larvae, the slow-growing clones disappeared from the wing disc. Since there was nothing wrong with the viability of the cells – flies of that genotype were viable - it appeared that they were eliminated due to interactions with wildtype cells in the same population. Cell competition is a homeostatic process that has recently attracted much attention for its role in removing undesirable and/or malignant cells from tissues in *Drosophila* and in vertebrates (reviewed in Baker, 2020; Vishwakarma and Piddini, 2020). For the history of cell competition see Morata, (2021).

6. Genetic basis of compartments

The existence of compartments as basic lineage units from early development provided a novel and unanticipated description of the body of *Drosophila*, which was also maintained in larval and imaginal structures. The first insights into the genetic basis of compartmentalization,

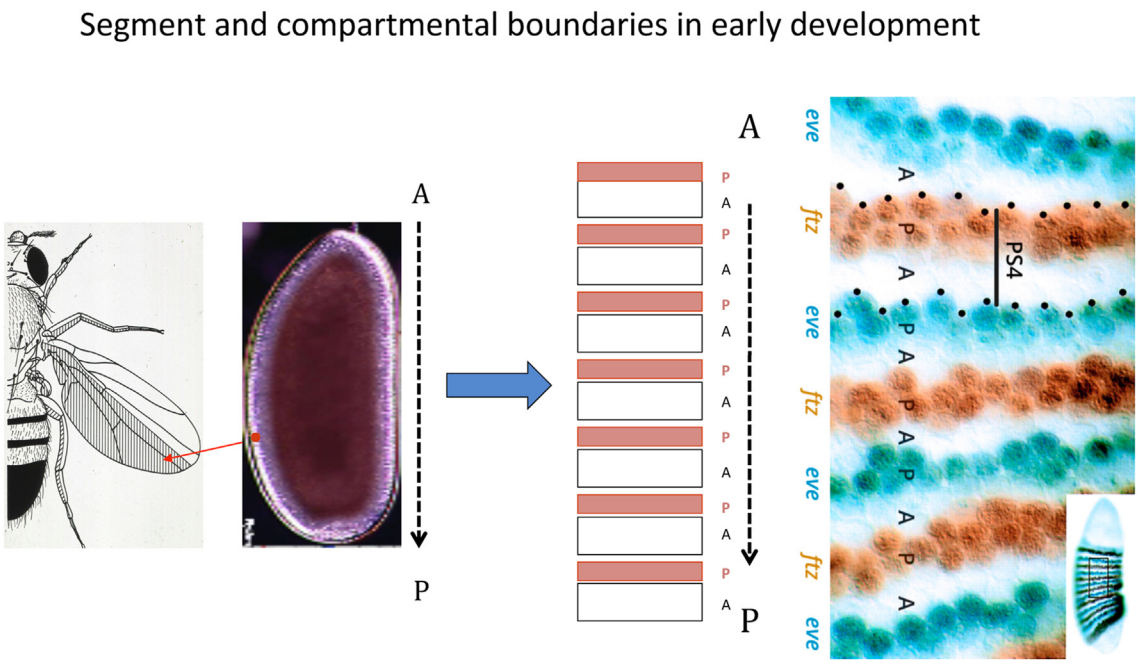

Fig. 3. Clonal analysis of early embryos. Marked clones induced at blastoderm (Wieschaus and Gehring *Dev. Biol* 1976; Lawrence and Morata, 1977) showed that the progeny of the clones was restricted to adult segments, and within segments it was also restricted to either A or P compartments, as illustrated by the drawing of the adult fly to the left. The red arrow shows the restriction of one individual blastoderm cell to form only P compartment structures. From the point of view of lineage the early embryo consists of a chain of A and P compartments along the anteroposterior body axis, represented to the right of the figure by the column of P (red) and A (white) blocks. This metameric organization can be visualized by the expression of early acting pair-rule genes likes *even-skipped* and *fushi tarazu,* which define the A/P boundaries (Lawrence et al., 1987). The image shows alternating stripes of cells expressing either *even-skipped* (blue) *or fushi tarazu* (brown). PS4 indicates the fourth parasegment (image from Lawrence and Struhl, 1996).

and by extension of the overall body organization, came from the wing disc, where the A/P line had been discovered in *Drosophila* (García--Bellido et al., 1973, 1976). Although similar, the A and P wing compartments show some morphological differences, especially in the bristles of the wing margins and in the vein pattern. Garcia-Bellido and Santamaria, (1972) had described a viable mutation named *engrailed*^{1} (*en*^{1}) that had abnormal wings in which the posterior region looked like a defective mirror-image copy of the anterior one. It raised the possibility that *en* could be required for the establishment of distinct anterior and posterior compartments in the wing. Work by Morata and Lawrence, (1975) demonstrated that it is indeed the case. There were two principal observations: 1) in *en*^{1} wings the A/P border is defective; using M^{+} clones it is not possible to delineate a strict lineage separation between the anterior and posterior regions (Fig. 4a), 2) the normal function of *en* is required only in the posterior compartment; mosaic analysis showed that *en*^{1} M^{+} clones in the anterior compartment have normal pattern and delineate a normal A/P boundary, but such mutant clones in the posterior compartment exhibit an anterior-like pattern and did not respect the A/P border (Fig. 4b). Moreover, *en* is required during the entire development of the disc, for even late induced *en*^{1} clones showed a posterior to anterior transformation. Later work showed that *en* is required in the posterior compartment of the eye-antenna (Morata et al., 1983), the abdomen (Kornberg, 1981b) and the proboscis disc (Struhl, 1981). The restriction of *en* activity to posterior compartments was later demonstrated directly by *in situ* hybridization of *en* transcripts (Kornberg et al., 1985) and by LacZ expression driven by the *en* promoter (Hama et al., 1990). Those studies established for the first time a connection between lineage units and gene function during development and also linked a specific gene function with particular regions of the body.

It took some time to find another gene involved in compartmentalization in the wing; Diaz-Benjumea and Cohen, (1993), identified the gene *apterous (ap)*, which with respect to the D/V border plays an equivalent role to that of *en* in the A/P border; *ap* is expressed only in the dorsal compartment where it is required to maintain the D/V border and to specify dorsal pattern. These results suggested the existence of a binary developmental code in the wing disc: *en* **on** would specify posterior and *en* **off** anterior compartment pattern, whereas *ap* would be **on** in the dorsal and **off** in the ventral compartment. The **on** and **off** combinations for *en* and *ap* would determine the specific development of four compartments in the wing disc.

Nevertheless, it has to be said that whereas the A/P border (and *en* function) is a general feature of the overall body design, present in all body parts and with versions in many animal groups, the D/V border has a more limited role; it appears during larval development and is only seen in the wing and possibly in haltere disc.

7. Developmental compartments in the interior of the fly, in the mesoderm and ectoderm?

All these experiments were restricted to the epidermis, so a marker was devised for the soft tissues (Lawrence, 1981) and what was found is entertaining. Yes the mesoderm is divided into metameric units by cell lineage (Lawrence, 1982) but these, unlike the ectoderm, are not further subdivided. And also these metameric units appear to be parasegments, not segments. There is even evidence that parasegments extend to the visceral mesoderm because there is metameric expression of those homeotic genes responsible for diversification of the ectoderm (see later here and Lawrence, 1992). By contrast, the endoderm is not metamerised by lineage and seems to have been inherited from unsegmented ancestors. The elemental idea that germ layers are more or less primitive and, and respectively, more or less sophisticated was made use of by Woody Allen in a diverting film about the functioning of the human body. https://www.imdb.com/title/tt0068555/

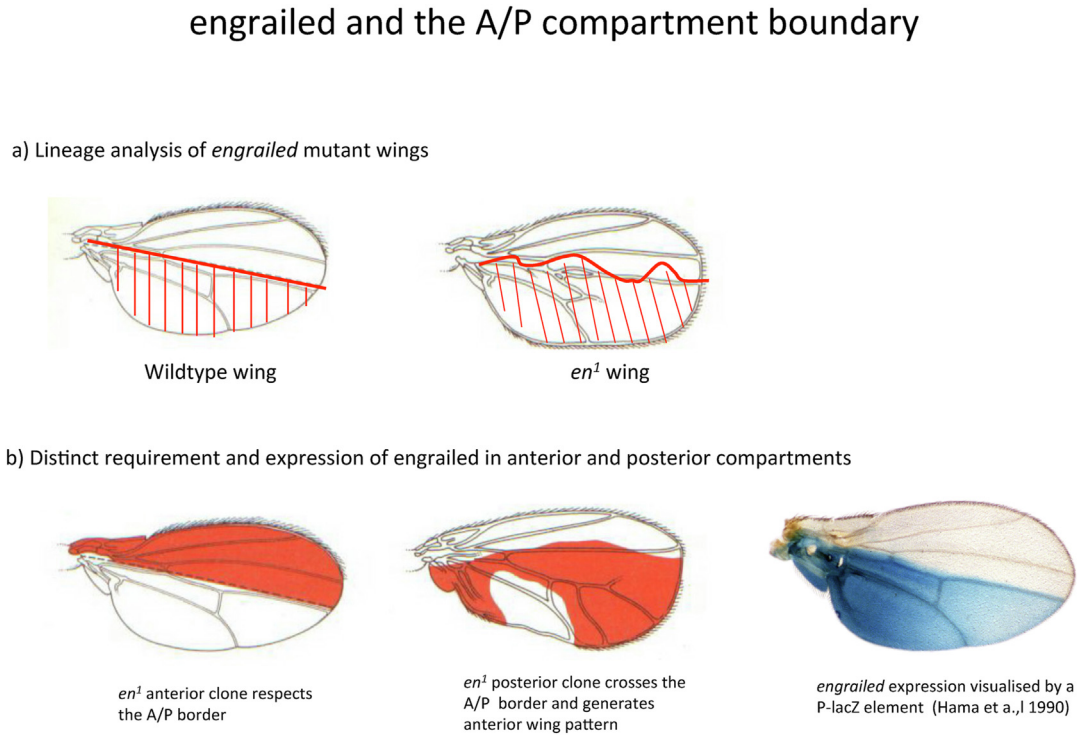

Fig. 4. a) Lineage analysis using the Minute technique of a *en*^{1} homozygous mutant wing (right) in comparison with a wildtype wing (left). In the *en*^{1} wing it is not possible to delineate a fixed A/P boundary.
b) Large *en*^{1} M^{+} clones (red) in the A compartment (left) do not affect the morphology of that compartment and also delineate the A/P boundary. Large *en*^{1} M^{+} clones in the P compartment (centre) alter the morphology of that compartment, which differentiates anterior patterns. These clones do not respect the A/P boundary and can penetrate into A compartment territory (Morata and Lawrence, 1975). The image on the right shows a wing showing the normal expression of *engrailed* as visualized by a P-lacZ element containing fragments of the *engrailed* promoter (Hama et al., 1990).

8. Compartments and morphogenesis

The discovery of compartments led to much research showing how the compartmental scaffold is instrumental in building pattern and form. In particular the A/P and D/V compartment boundaries are the sites from where the major morphogens originate. The functions of the morphogens Hedgehog and Decapentaplegic are associated with the A/P border and that of Wingless with the D/V one. These boundaries are the anatomical references that establish positional information and determine the pattern and growth of the wing (and other) discs (Nellen et al., 1996; Lecuit et al., 1996, reviewed in Lawrence and Struhl, 1996). Further analysis of their function continues to this day and is beyond our scope.

9. The formation of the metameric scaffold

As mentioned above, the anteroposterior axis of the *Drosophila* embryo consists of chain of parasegments, and these later resolve into a chain of P-A-P-A compartments.

How does the original zygote generate this pattern? This was the question addressed by Christiane Nusslein-Volhard and Eric Wieschaus, who investigated the genetic basis of fly development. They set up a screen to isolate mutations that alter the metameric organization of the body. This screen could not be performed with adult flies, for the majority of the mutations (around 90%) are adult lethal, but they realized that most of the lethal mutants develop larval patterns, although many do not hatch from the egg. Therefore they used the late embryonic stage to assay the phenotype of new mutations affecting segmentation. In retrospect, this shifting of attention from adult to larval patterns was of enormous importance because at one stroke Nusslein-Volhard and Eric Wieschaus opened up 90% of the *Drosophila* genome for investigation.

As the aim of the experiment was to identify all the genes affecting segmentation, it was critical that the experiment was to be completed to "saturation", that is, until all the candidate genes had been identified. It required the isolation of several mutations per gene, making it unlikely that there are genes that have not been mutated.

Unexpectedly, after amassing a large number of new mutations, they identified only 15 complementation groups that specifically affected segmental patterns (Nusslein-Volhard and Wieschaus, 1980). The implication was that the genetic analysis of the larval segmentation was not, as one might have imagined, impossibly complex. Moreover, the phenotypes of these mutations could be placed into classes: gap, pair-rule and polarity, what already suggested different steps in the process. A first subdivision into large body regions, followed by the formation of double segment units, and finally individual segments. Since it was likely that the genes controlling larval and adult segmentation were the same, this study provided much insight into the body design.

The impact of the paper by Nusslein-Volhard and Wieschaus cannot be overemphasized. It identified all (or the majority of) the genes responsible for the larval pattern and provided mutations of them. Some of these genes established a direct connection with the A/P boundary (the major factor responsible for the metameric organization); for example Lawrence et al., (1987) found that the delimitation of the A/P borders is established during embryogenesis by *fushi tarazu* and *even-skipped*, two of the pair-rule genes identified in the screen (Fig. 3).

Moreover, their paper appeared just at the time when molecular techniques for cloning DNA had been developed. The availability of the new mutations allowed the cloning of these genes and soon that was achieved. This was the beginning of genetic and molecular analysis of pattern formation in development.

10. Morphological diversity along the anteroposterior body axis. The homeotic genes

Through the activity of maternal and segmentation genes, after a few hours of development, the *Drosophila* embryo has acquired a metameric organization, a chain of segmental units (A-P, or P-A). Putting aside the pole cells, located at the posterior end, all metameres look remarkably similar at that early stage (Fig. 3).

10.1. The homeotic genes. The BX-C and the ANT-C

The term homeosis was introduced in the XIX century to describe some rare cases of organisms in which a part of the body appeared transformed to resemble a different part (Bateson, 1894). Although homeotic transformations had been described in a number of species, it is in insects and particularly in *Drosophila* where homeosis has been extensively studied. Classical examples of homeosis are the *Antp* mutations in which the antennae are transformed into legs or the mutations at the bithorax genes in which halteres were transformed into wings (Fig. 5).

A pioneer in the study of homeotic genes in *Drosophila* was Edward Lewis, who for many years studied the mutations of the Bithorax (Bx) system (Lewis, 1951, 1963, 1978). This system fell in the category of "pseudoallelic series", groups of clustered genes, related functionally but not identical (Lewis, 1951). Viable mutations called *bithorax (bx)* and *postbithorax (pbx)* caused transformations of the third thoracic segment (T3), which includes the haltere and the third leg, into the second thoracic segment (T2), which includes the wing and the second leg. The *bithoraxoid (bxd)* mutation transformed the first abdominal (A1) segment into T3. The transformations caused by these mutations affected single segments, but intriguingly the *bx* and *pbx* transformations affected exclusively either the anterior (*bx*) of the posterior (*pbx*) regions of the haltere. The significance of this observation was only appreciated when compartments were discovered. The genetics of the Bx system was complicated and included other mutations, like *Ultrabithorax* (*Ubx*), with a complex phenotype that included those of the *bx*, *pbx* and *bxd* mutations. Based on the phenotypes of those mutations, the realm of action of the Bx system would be the T3 and the A1 segments.

A big change in understanding of the Bx system, as it was then called, came after Lewis studied a deletion (called P9) that lacked all the Bx genes. The P9 deletion is homozygous lethal so the adult phenotypes could not be studied, but P9 embryos secrete larval cuticle and the larval patterns could be examined. Lewis, (1978) found that, in P9 larvae, all segments from T3 to the last abdominal one (A8) developed alike with a thoracic-like pattern (Fig. 6a). At one stroke the realm of action of the Bx genes was revealed to extend to the whole of the abdomen. The result implicated the existence of genes, yet undiscovered, in charge of the development of the abdominal segments. The bithorax system was upgraded and became called the bithorax complex (BX-C).

Based on the logic dictated by the viable mutations, which appeared to affect individual segments, Lewis, (1978) proposed a model of BX-C organization in each gene would determine the development of a specific segment; a one gene/one segment model (Fig. 6b). After the finding that the limits of expression of some Bx genes were not segment borders but A/P compartment boundaries (Morata and Kerridge, 1981; Struhl, 1984), Struhl proposed the "out-of-register" model, also a one gene/one metamere model, but in which the activity of the Bx genes was shifted by one compartment, meaning they defined parasegmental boundaries (Fig. 6b). Both models predicted the existence of 8–9 BX-C genes but the elusive BX-C abdominal genes had yet to be identified. The cloning of the BX-C (Bender et al., 1983; Karch et al., 1985) provided for the first time a molecular description of the different mutants.

The Morata group (Sánchez-Herrero et al., 1985) tackled this problem with a classical genetic approach: they used saturation mutagenesis of the P9 deletion to find out how many complementation groups it comprises. Surprisingly, only three complementation groups were found, one corresponding to the *Ubx* gene, already known, and two others, *abdominal-A (abd-A)* and *Abdominal-B (Abd-B),* which would be responsible for the development of the abdominal segments. The triple mutant combination for these genes yielded the same phenotype as the P9 deletion (Casanova et al., 1987), demonstrating that the three genes comprised all the BX-C functions. These results strongly argued against the one-gene/one-metamere models and implied that the realm of action of

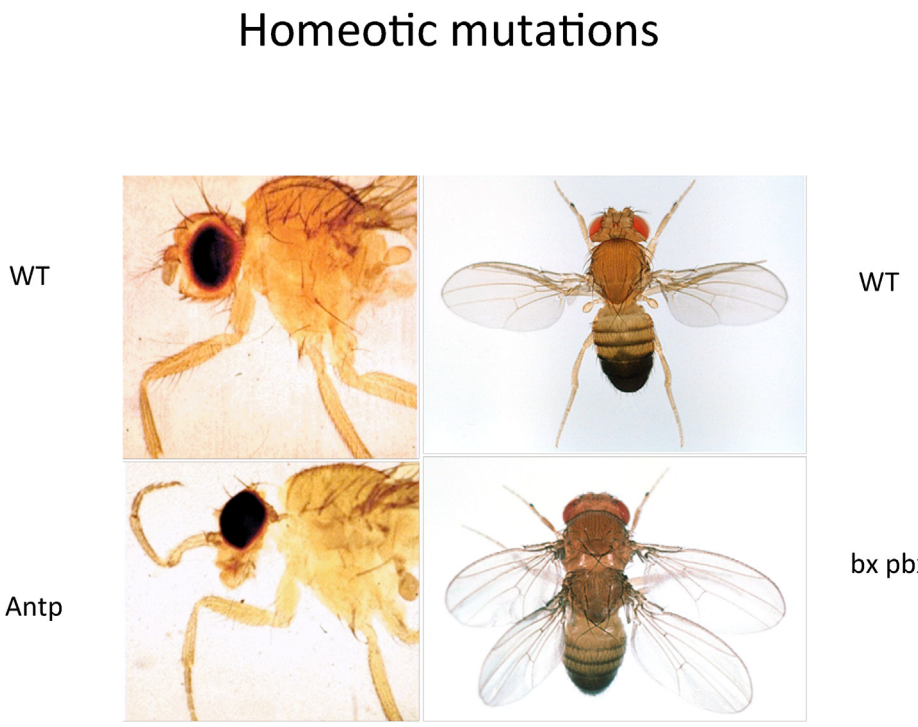

Fig. 5. Phenotypes of two classical homeotic mutations, *Antp* and the *bx pbx* mutant combination. The images on top show a wildtype flies. The bottom image (left) shows an Antp mutant in which the antenna is replaced by a supernumerary leg. The antennal leg is of normal morphology. In a *bx pbx* mutant fly (right) the metathoracic segment, which normally includes the metanotum and the haltere, develop instead mesothoracic structures, notum and wings. As a result the *bx pbx* fly contains four wings and no halteres. Note that additional wings and notum are perfectly formed, indicating that the lack of *bx pbx* functions drives the metathoracic cells to adopt a normal pattern, but one that does not correspond to the position.

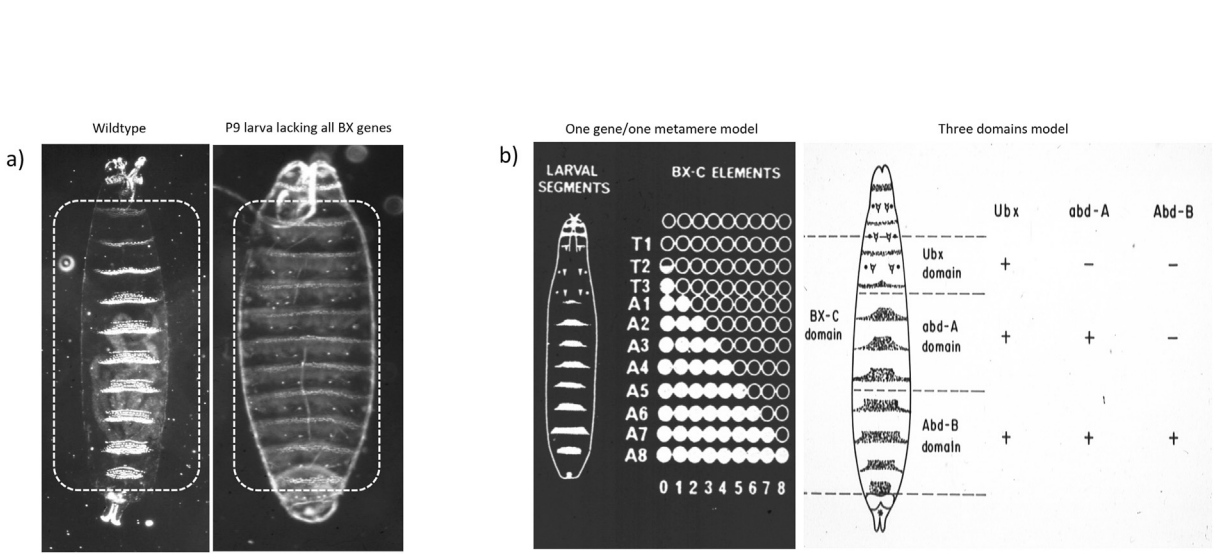

Fig. 6. a) The images illustrate the implications of the discovery by Ed Lewis that embryos homozygous for the P9 deletion, lacking all the BX-C genes, show a transformation of the metathoracic and all the abdominal segments, each of which develops a mesothoracic-like pattern. The body region affected is delineated by the white dotted lines and represents the entire realm of action of the BX-C. The fact that the phenotype of P9 extends to the whole abdomen implied that the BX-C must include genes responsible for the development of all abdominal segments. b) The two models proposed to explain the deployment of BX-C genes along the anteroposterior body axis. In the one gene/one metamere models (Lewis, 1978; Struhl, 1984) the development of each segment (or parasegment) would be determined by a specific combination of BX-C genes, in which the key role is exerted by a more posterior acting gene (solid circles). T3 development requires one gene (*Ubx* in the model) activity, whereas A1 would be specified by the *Ubx* and the A1 specific gene, *bxd* in this case. Subsequent abdominal segments would be specified by additional abdominal genes, also segment (or parasegment)-specific. Ultimately those models implied the existence of 9 BX-C genes.
In the three domains model (Sánchez-Herrero et al., 1985) each of the three BX-C genes plays a major role in a specific domain. The Ubx domain is specified solely by *Ubx*, the abd-A domain by *Ubx* and *abd-A* and the Abd-B domain by *Ubx*, *abd-A* and *Abd-B*. The extent of each of the three domains is delimited by A/P compartment boundaries, as indicated by the horizontal dotted lines in the image to the right.

the BX-C only comprises three body domains (Fig. 6b). The three domain model by the Morata group was counterintuitive and caused some controversy, for it was not clear how each individual BX-C gene could control the development of several different segments, but the finding that there are only three homeoboxes (see later) in the complex as well as the transcript analysis (Regulski et al., 1985) gave strong support to the

model. It became clear that some of the genes proposed by Lewis were cis regulatory elements of the complex. In the case of *Ubx*, Cabrera et al., (1985) showed that the *bx* and *pbx* mutations suppressed the expression of *Ubx* in the haltere disc. The finding of homologs of *Ubx*, *abd-A* and *Abd-B* in many other species, including mammals, definitely settled the issue.

The BX-C accounts for the development of part of the thorax and the abdomen of the body, but not for the more anterior body regions. This role is fulfilled by another cluster of homeotic genes, those of the Antennapedia Complex (ANT-C). It contains five genes each with homeobox*, labial, proboscipedia, Deformed, Sex combs reduced* and *Antennapedia*, whose functions control the identity of cephalic and anterior (T1 and T2) thoracic segments. Work from the Kaufman group reported the phenotypes of the ANT-C mutants (Wakimoto and Kaufman, 1981) as well as the molecular organization of the complex (Scott et al., 1983; Kaufman et al., 1990). Interestingly, unlike the BX-C, the ANT-C includes genes like *fushi tarazu*, *bicoid, zerknüllt (zen)* and *zerknüllt-2 (z-2)* that play important developmental roles but are not homeotic in character.

The activities of the ANT-C and BX-C would account for the development of all fly segments, except for the analia, located at the posterior end (Fig. 7). The homeotic gene responsible for analia development was later identified as *caudal* (Moreno and Morata, 1999), thus completing the genetic catalogue for the entire body (Fig. 7). The clustering of the ANT-C and the BX-C genes and the similarity of their developmental roles strongly suggested a common evolutionary origin.

10.2. The homeobox discovery

Fascinating as the homeotic genes were, it was not understood how a sole gene function, say *Ubx*, could control the development of a whole region of the fly, in this case both the T3 and the A1 segments. One possibility was that homeotic genes would regulate the activity of subsidiary genes that would be responsible for the individual characteristics and morphology (identity) of the corresponding body part, but there was no evidence for this. The discovery of the homeobox shed light on this and other features of the homeotic genes.

The advent of molecular techniques in the early eighties allowed the first molecular descriptions of homeotic genes. Two key papers, from the Gehring (McGinnis et al., 1984b) and Scott (Scott and Weiner, 1984) groups found that several homeotic genes contained a short (180) stretch of basepairs in common, which is referred to as homeobox.

The discovery of the homeobox had an enormous impact in the field and particularly in the understanding of the function of the homeotic genes. First, the presence of the homeobox in all the ANT-C and BX-C genes confirmed their common evolutionary lineage. It also indicated that they have appeared by successive tandem duplications, which resulted in their localization in clusters. The term HOM-C was coined (Akam, 1984) to refer jointly to the ANT-C and the BX-C.

Second, the homeobox encodes a DNA binding protein domain, clearly pointing to a molecular mechanism of homeotic function: these genes are master regulatory factors that regulate many other subsidiary (downstream) genes.

Third, the homeobox sequence could be used as a molecular probe to search for homeotic genes in other species, humans for example, in which their identification by genetic methods would be hard or impossible. It was immediately found that homeobox-containing genes are present in different animal groups (McGinnis et al., 1984a; Carrasco, 1984), indicating that the homeobox is a general feature of all metazoans. Moreover, not only are the homeobox-containing genes conserved, but the structure of the complex, the clustering of the genes and their order, is also preserved (Duboule and Dolle, 1989; Graham et al., 1989). After the demonstration of their presence in many animal groups, the term Hox was introduced to designate these genes. Overall, these findings provided a general image of the genetic design of the metazoan body (McGinnis and Krumlauf, 1992).

The Cold Spring Harbor meeting in 1985 was a key event in this period of research. We remember it as a fun meeting containing several cultural "interactions" between the US and European perspectives of developmental biology. Looking back we can now see that what was found in *Drosophila* took a long time to influence the mainstream, which of course was centered on vertebrates. That conquest began with rhombomeres, but another lesson from *Drosophila* lineage studies is that the description of vertebrate embryos as divided into germ layers should be a cell lineage or "compartmental" concept. A view that was neglected because compartments were late in making their debut and were up against the traditional and almost mystical significance given to the germ

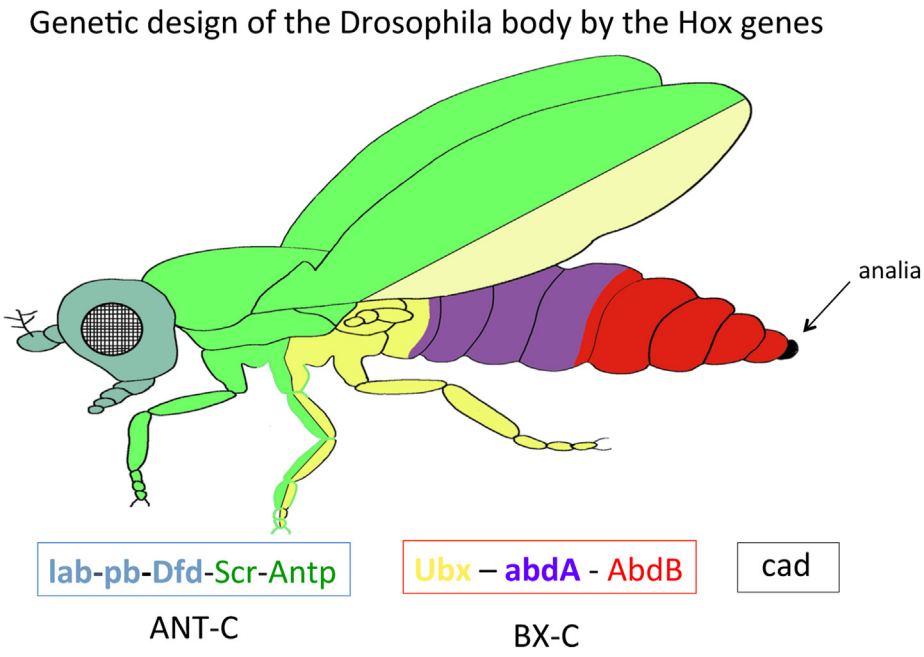

Fig. 7. Simplified scheme of the genetic design of the *Drosophila* body along the anteroposterior axis. The development of the anterior body regions is determined by the genes of the Antennapedia Complex (ANT-C), *labial (lab), proboscipedia (pb), Deformed (Dfd), Sex comb reduced (Scr)* and *Antennapedia* (*Antp*) and *caudal (cad)*. Defining the expression domains of each of the ANT-C genes is hampered by the invagination of the head segments during embryogenesis. The BX-C genes determine the development of the posterior part of the body, from the A/P border of the mesothoracic to the last abdominal segment A8. The expression domains of the BX-C genes are labelled in colour. Note that the most posterior body region, the analia (arrow) is not specified by ANT-C or the BX-C genes. Work by Moreno and Morata, (1999) showed that analia development is specified by the gene *caudal*, another homeobox-containing gene, located elsewhere in the genome.

layer concept. And thus it is still not fully recognized today that the most objective way to define germ layers was and is to do cell marking experiments. An amusing example of the power of this approach came a little later from the Malpighian tubules of *Drosophila* (Denholm et al., 2003), where it was found by lineage studies that these tubules are made from two different types of cells, one ectodermal and one mesodermal, the latter migrating in to join the former. We think that more marking experiments in vertebrates could still prove very illuminating.

References

Akam, M., 1984 A. Common segment in genes for segments of Drosophila. Nature 308, 402–403. https://doi.org/10.1038/308402a0.PMID:6424024.

Baker, N.E., 2020. Emerging mechanisms of cell competition. Nat. Rev. Genet. 21, 683–697. https://doi.org/10.1038/s41576-020-0262-8. Epub 2020 Aug 10.

Bateson, W., 1894. Materials for the Study of Variation Treated with Especial Regard to Discontinuity in the Origin of Species. MacMillan and Co.

Beadle, G.W., Ephrussi, B., 1936. The Differentiation of Eye Pigments in Drosophila as Studied by Transplantation Genetics, vol. 21, pp. 225–247.

Bender, W., et al., 1983. Molecular Genetics of the Bithorax Complex in Drosophila melanogaster Science, vol. 221, pp. 23–29. https://doi.org/10.1126/science.221.4605.23.

Bryant, P.J., 1970. Cell lineage relationships in the imaginal wing disc of Drosophila melanogaster Develop. Biol. 22, 389–411. https://doi.org/10.1016/0012-1606(70)90160-0.

Bryant, P.J., 1975. Pattern formation in the imaginal wing disc of Drosophila melanogaster: fate map, regeneration and duplication. J. Exp. Zool. 193, 49–77. https://doi.org/10.1002/jez.1401930106.

Cabrera, C.V., Botas, J., Garcia-Bellido, A., 1985. Distribution of Ultrabithorax protein in mutants of Drosophila bithorax complex and its transregulatory genes. Nature 318, 569–571.

Carrasco, AE, et al., 1984. Cloning of a X. Laevis gene expressed during early embryogenesis coding for a peptide region homologous to Drosophila homeotic genes. Cell 37, 409–414. https://doi.org/10.1016/0092-8674(84)90371-4.PMID:6327066.

Casanova, J., Sánchez-Herrero, E., Busturia, A., Morata, G., 1987. Double and triple mutant combinations of the bithorax complex genes of *Drosophila*. EMBO J. 6, 3103–3109.

Crick, F.H., Lawrence, P.A., 1975. Compartments and polyclones in insect development. Science 189, 340–347.

Denholm, B., et al., 2003. Dual origin of the renal tubules in Drosophila: mesodermal cells integrate and polarize to establish secretory function. Curr. Biol. 13, 1052–1057. https://doi.org/10.1016/s0960-9822(03)00375-0.

Diaz-Benjumea, F.J., Cohen, S.M., 1993. Interaction between dorsal and ventral cells in the imaginal disc directs wing development in. Drosophila Cell 75, 741–752.

Duboule, D., Dolle, P., 1989. The structural and functional organization of the murine Hox gene family resembles that of Drosophila homeotic genes. EMBO J. 8, 1497–1505.

Fraser, S., Keynes, R., Lumsden, A., 1990. Segmentation in the chick hindbrain is defined by cell lineage restrictions. Nature 344, 431–435. https://doi.org/10.1038/344431a0.

Garcia-Bellido, A., Merriam, J.R., 1971. Parameters of the wing imaginal disc development of Drosophila melanogaster Develop. Biol. 24, 61–87. https://doi.org/10.1016/0012-1606(71)90047-9, 1971.

Garcia-Bellido, A., Santamaria, P., 1972. Developmental Analysis of the Wing Disc in the Mutant Engrailed of Drosophila melanogaster Genetics, vol. 72, pp. 87–104. https://doi.org/10.1093/genetics/72.1.87.

García-Bellido, A., Ripoll, P., Morata, G., 1973. Developmental compartmentalization of the wing disc of *Drosophila*. Nat. New Biol. 245, 251–253.

García-Bellido, A., Ripoll, P., Morata, G., 1976. Developmental segregations in the dorsal mesothoracic disk of *Drosophila*. Devel. Biol. 48, 132–147.

Gehring, W., 1967. Clonal analysis of determination dynamics in cultures of imaginal disks in Drosophila melanogaster Develop. Biol. 16, 438–456.

Gehring, W., 1972. The stability of the determined state in cultures of imaginal disks in Drosophila. In: Nöthiger, R., Ursprung, H. (Eds.), The Biology of Imaginal Disks. Heidelberg: Springer Berlin Heidelberg, Berlin, pp. 35–58.

Graham, A., Papalopulu, N., Krumlauf, R., 1989. The murine and Drosophila homeobox gene complexes have common features of organization and expression. Cell 57, 378–387.

Gurdon, J, 1988. A community effect in animal development Nature. Nature 336, 772–774. https://doi.org/10.1016/0012-1606(81)90347-x.PMID: 6780397.

Hadorn, E., 1968. Transdetermination in cells. Sci. Am 219, 110–114. https://doi.org/10.1038/scientificamerican1168-110. PMID: 5684924.

Hama, C., Ali, Z., Kornberg, T.B., 1990. Region-specific recombination and expression are directed by portions of the *Drosophila engrailed* promoter. Genes Dev. 4, 1079–1093.

Karch, F., et al., 1985. The Abdominal Region of the Bithorax Complex Cell, vol. 43, pp. 81–96. https://doi.org/10.1016/0092-8674(85)90014-5.

Kaufman, T.C., Seeger, M.A., Olsen, G., 1990. Molecular and genetic organization of the Antennapedia gene complex of Drosophila melanogaster. In: Wright, P.R.F. (Ed.), Advances in Genetics: Genetic Regulatory Hierarchies in Development, vol. 27, pp. 309–382.

Kornberg, T., 1981a. Compartments in the abdomen of Drosophila and the role of the engrailed locus Develop. Biol. 86, 363–372.

Kornberg, T., 1981b. Engrailed: a Gene Controlling Compartment and Segment Formation in Drosophila Proc Natl Acad Sci U S A. 1981. PMID: 6821526.

Kornberg, T., Siden, I., O'Farrell, P., Simon, M., 1985. The engrailed locus of Drosophila: in situ localization of transcripts reveals compartment-specific expression. Cell 40, 45–53. https://doi.org/10.1016/0092-8674(85)90307-1.

Lawrence, P.A., 1973. A clonal analysis of segment development in Oncopeltus (Hemiptera). J. Embryol. Exp. Morphol. 30, 681–699.

Lawrence, P.A., 1981. A general cell marker for clonal analysis of Drosophila development. J. Embryol. Exp. Morphol. 64, 321–332.

Lawrence, P.A., 1982. Cell lineage of the thoracic muscles of Drosophila. Cell 29, 493–503.

Lawrence, P.A., 1992. The Making of a Fly: the Genetics of Animal Design. Blackwell Scientific.

Lawrence, P.A., Morata, G., 1977. The early development of mesothoracic compartments in *Drosophila*. Develop. Biol. 56, 40–51.

Lawrence, P.A., Struhl, G., 1996. Morphogens, compartments and patter: lessons from Drosophila? Cell 28, 951–961. https://doi.org/10.1016/s0092-8674(00)81297-0.

Lawrence, P.A., Johnston, P., Morata, G., 1986. Methods of marking cells. In: Roberts, D.B. (Ed.), Drosophila: A Practical Approach. IRL Press Oxford and Washington, pp. 229–242.

Lawrence, P.A., Johnston, P., Macdonald, P., Struhl, G., 1987. Borders of parasegments in Drosophila embryos are delimited by the fushi tarazu and even-skipped genes. Nature 328, 440–442. https://doi.org/10.1038/328440a0.

Lecuit, T., Brook, W.J., Ng, M., Calleja, M., Sun, H., Cohen, S.M., 1996. Two distinct mechanisms for long-range patterning by Decapentaplegic in the Drosophila wing. Nature 381, 387–393.

Lewis, E.B., 1951. Pseudoallelism and gene evolution. Cold Spring Harbor Symp. Quant. Biol. 16, 159–174.

Lewis, E.B., 1963. Genes and developmental pathways. Am. Zool. 3, 33–56.

Lewis, E.B., 1978. A gene complex controlling segmentation in Drosophila. Nature 276, 565–570.

Lindsley, D., Grell, E., 1968. Genetic Variations in Drosophila. Carnegie Inst. Washington, Washington, D.C., U.S.A, p. 469.

Madhavan, M.M., Schneiderman, H.A., 1977. Histological analysis of the dynamics of growth of imaginal discs and histoblast nests during the larval development of Drosophila melanogaster. Wilhelm Roux Archiv 183, 269–305.

Martín, F.A., Herrera, S.C., Morata, G., 2009. Cell competition, growth and size control in the Drosophila wing imaginal disc. Development 136, 3747–3756.

Martinez-Arias, A., Lawrence, P.A., 1985. Parasegments and compartments in the Drosophila embryo. Nature 313, 639–642.

Marygold, S.J., Roote, J., Reuter, G., Lambertsson, A., Ashburner, M., et al., 2007. The ribosomal protein genes and Minute loci of Drosophila melanogaster. Genome Biol. 8, R216. https://doi.org/10.1186/gb-2007-8-10-r216.

McGinnis, W., Krumlauf, R., 1992. Homeobox genes an axial patterning. Cell 66, 263–602.

McGinnis, W., Garber, R.L., Wirz, J., Kuroiwa, A., Gehring, W.J., 1984a. A Homologous Protein-Coding Sequence in Drosophila Homeotic Genes and its Conservation in Other Metazoans Cell, vol. 37, pp. 403–408. https://doi.org/10.1016/0092-8674(84)90370-2.

McGinnis, W., Levine, M.S., Hafen, E., Kuroiwa, A., Gehring, W.J., 1984b. A conserved DNA sequence in homeotic genes of the Antennapedia and bithorax complexes. Nature 308, 428–433. https://doi.org/10.1038/308428a0.

Morata, G., 2021. Cell competition: a historical perspective. Dev. Biol. 476, 33–40.

Morata, G., Kerridge, S., 1981. Sequential functions of the bithorax complex of Drosophila. Nature 290, 778–781.

Morata, G., Lawrence, P.A., 1975. Control of compartment development by the engrailed gene in Drosophila. Nature 255, 614–617.

Morata, G., Lawrence, P.A., 1978. Anterior and posterior compartments in the head of Drosophila. Nature 274, 473–474.

Morata, G., Ripoll, P., 1975. Minutes: mutants of drosophila autonomously affecting cell division rate. Develop. Biol. 42, 211–221.

Morata, G., Kornberg, T., Lawrence, P.A., 1983. The phenotype of *engrailed* mutations in the antenna of *Drosophila*. Develop. Biol. 99, 27–33.

Moreno, E., Morata, G., 1999. Caudal is the Hox gene that specifies the most posterior Drosophila segment. Nature 400, 873–877. https://doi.org/10.1038/23709.

Nellen, D., Burke, D., Struhl, G., Basler, K., 1996. Direct and long-range action of a DPP morphogen gradient. Cell 85, 357–368.

Nusslein-Volhard, C., Wieschaus, E., 1980. Mutations affecting segment number and polarity in Drosophila. Nature 287, 795–801. https://doi.org/10.1038/287795a0.

Regulski, M., Harding, K., Kostriken, K., Karch, F., Levine, M., McGinnis, W., 1985. Homeo box genes of the Antennapedia and bithorax complexes of Drosophila. Cell 43, 71–80.

Sánchez-Herrero, E., Vernos, I., Marco, R., Morata, G., 1985. Genetic organization of *Drosophila* bithorax complex. Nature 313, 108–113.

Scott, M., Weiner, A.J., 1984. Structural relationships among genes that control development: sequence homology between the Antennapedia, Ultrabithorax, and fushi tarazu loci of Drosophila. Proc. Natl. Acad. Sci. U. S. A. 81, 4115–4119.

Scott, M.P., Weiner, A.J., Hazelrigg, T.I., Polisky, B.A., Pirrotta, V., Scalenghe, F., Kaufman, T.C., 1983. The Molecular Organization of the Antennapedia Locus of Drosophila Cell, vol. 35, pp. 763–776. https://doi.org/10.1016/0092-8674(83)90109-5.

Steiner, E., 1976. Establishment of compartments in the developing leg imaginal discs of Drosophila melanogaster Wilhelm Roux. Arch. Plus 180, 9–30.

Struhl, G., 1981. Anterior and posterior compartments in the proboscis of Drosophila 1981 Develop. Biol. 84, 372–385. https://doi.org/10.1016/0012-1606(81)90406-1.
Struhl, G., 1984. Splitting the Bx complex Nature 308, 454–457.
Vishwakarma, M., Piddini, E., 2020. Outcompeting cancer. Nat. Rev. Cancer 20, 187–198. https://doi.org/10.1038/s41568-019-0231-8. Epub 2020 Jan 13.
Wakimoto, BT, Kaufman, TC, 1981. Analysis of larval segmentation in lethal phenotypes associated with the antennapedia gene complex in Drosophila melanogaster. Develop. Biol 81, 51–64. https://doi.org/10.1016/0012-1606(81)90347-x.PMID: 6780397.
Wieschaus, E., Gehring, W., 1976. Clonal analysis of primordial disc cells in the early embryo of Drosophila melanogaster. Develop, Biol. 50, 249–263.