



DROSOPHILA MELANOGASTER RESEARCH: HISTORY, BREAKTHROUGH AND PERSPECTIVES

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Abstract

The common fruit fly, or *Drosophila Melanogaster*, has been used as an object of biomedical studies for over a century. It has been mostly employed in genetic research, as it exhibits several advantages which make its use relatively easy and cheap, with the results widely translatable into further vertebrate studies. This model has been the basis of the work of Christiane Nüsslein-Volhard, who together with Eric Wieschaus unravelled much of the mystery surrounding early *drosophila* development in the 1970s-1980s, laying foundations for broader understanding of multicellular organism embryogenesis, which brought them a Nobel prize in Physiology and Medicine in 1995. The knowledge gained from *drosophila* studies improves the basic understanding of developmental processes, while the model itself is relatively easy to maintain, analyse and translate the results onto other species. While models such as Zebrafish present better with other vertebrates, *drosophila* remains a very important element of genetic research, finding even more applications with the development of current science and medicine. Hence, in this short review, the outline of the history, breakthroughs and perspectives of the *drosophila* research has been presented.

Running title: Factors of pituitary and hypothalamus development

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Drosophila Melanogaster as a model animal

The common fruit fly, or *Drosophila Melanogaster*, has been used as an object of biomedical studies for over a century. Its use was popularised by Thomas Hunt Morgan, who used the model to create the definition of genes, as well as establish their location on the chromosomes, which brought him a Nobel Prize in Physiology and Medicine. The fruit fly was further used by his student, Herman Muller, who discovered the adverse effects of radiation on the integrity of the genome, which also yielded him a Nobel Prize. Since then, the *Drosophila* model has been widely used in genetic research, as it exhibits several advantages which make its use relatively easy and cheap, with the results widely translatable into further vertebrate studies. This is especially important, as due to the long generation time, as well as obvious ethical constraints, genetic studies conducted in humans, particularly those concerning the early developmental processes, are impossible to perform [1].

Using *drosophila model* overcomes those troubles through several of its properties. Firstly, the fruit flies can be bred in large numbers, as each female is able to lay up to around a 100 eggs each day, for as long as 20 days. Secondly, their whole developmental process occurs over a relatively short period (around 10 days at 25°C from an embryo to the fully adult fly), which allow for easy observation of phenotypes resulting from particular gene manipulation. Thirdly, the process of individual fly manipulation, during processes such as selective cross-breeding, is also easily conductible, as the flies can be anesthetized with carbon dioxide or ether, analysed under low magnification microscope or magnifying glass, and moved around using a fine tipped brush, all without major effort. Finally, the fruit fly doesn't require any expensive housing conditions or specific feed, as multiple specimen can be easily kept in standard conical flasks secured with cotton plug, feeding on a jelly mix placed at the bottom of the flask (made of water, cornmeal, yeast, soy flour, malt extract, corn syrup and agar) [2].

The life cycle of the *Drosophila* is quite distinct from that of vertebrate species. The fruit fly develops in four stages. The first stage, the egg, lasts just around one day after fertilisation. Afterwards, a larva hatches, spending the next five days of development on continuous feeding and growth. Then, the pupation occurs, lasting around four days, resulting in a fully mature fly. The majority of tissues proprietary to the early embryo and the larva are discarded during the pupa stage. The complete adult organism develops from a conserved populations of cells known as imaginal discs, induced in the early embryonic development [3]. However, despite the fact that these processes seem nothing alike to those occurring in the human organism, the *Drosophila* model has been successfully employed in a range of

research, with results that turned out to be highly translatable across the species.

Drosophila melanogaster has been primarily used for research considering the influence of different genes in various biological processes. There are several reasons for that, which make the fruit fly a genetic model of potential incomparable to any other multicellular organism. The model has been studied for over the century, with even the earliest research resulting in discovery of some genes that are now found to be immensely important. As was mentioned before, the extremely short generation time allows to easily cross the specimen, and observe the phenotype of the offspring in a relatively short time. The discovered genes are easily described using nomenclature relating to the phenotypical results of their mutations. This tradition dates to the initial research by Thomas Hunt Morgan, resulting in gene names such as *wingless* (mutant flies lack wings), *Notch* (mutants have notches on their wings), or *groucho* (mutant flies exhibit appearance that, according to the researchers, resembled the famous comedian Groucho Marx). Relative ease in observing and describing phenotypes of the mutation affected fruit flies allows for a research method that involves exposing the specimen to mutagens and analysing the genomes of abnormal specimen, identifying the genes responsible for the specific mutations [1].

Drosophila Genetics and Christiane Nüsslein-Volhard

This approach been the basis of the work of Christiane Nüsslein-Volhard, who together with Eric Wieschaus unravelled much of the mystery surrounding early *drosophila* development in the 1970s-1980s, laying foundations for broader understanding of multicellular organism embryogenesis.

However, the first stage of her research, conducted at the University of Basel, Switzerland, concerned studies on the effects of the mutations of the *bicaudal* gene on embryo polarity. Knowledge on the role of this gene, back then recently described as crucial for early embryo patterning [4], still did not fully describe the conditions needed for complete dorsal-ventral alignment. Its mutations cause characteristic phenotypes of the affected embryos, manifesting in duplication of the posterior abdominal segments along the longitudinal axis, as well as lack of the anterior body segments (thorax, head). The study analysed the offspring of the cross-breeding of flies known previously produce embryos characterised with a phenotype associated with mutation in the *bicaudal* gene. An analysis of a large amount of the resulting offspring was conducted, with different severity of the *bicaudal* mutation effect observed. Some of the embryos exhibited normal polarity and fully developed head. However, they lacked non-terminal segments, with abdominal or thoracic parts failing to develop. Most of the em-

bryos exhibited bicaudal phenotypes of ranging severity, from those of symmetrically duplicated caudal projections, to some that, despite duplication, lacked some critical anterior or posterior structures. Finally, a small amount of offspring exhibited relatively normal symmetry, in some accompanied by head abnormalities, or did not develop past the egg stage. The analysis of the distribution of the various phenotypes allowed to identify that *bicaudal* is a hypomorphic (begins to manifest when the gene function drops below necessary threshold) maternal-effect mutation (as the paternal genotype does not affect the frequency of the mutant phenotype appearance), with recessive inheritance pattern [5,6]. Additionally, the prevalence of mutant embryos was negatively correlated with the increase in maternal age and positively correlated with increase in temperature in which the flies are kept. The results were discussed, emphasizing essential role of *bicaudal* in the initial patterning of the *Drosophila* embryos. The discussion also suggests presence of more factors that work in a manner of gradient pattern between the anterior and posterior terminus of the embryo, to ensure the proper axis formation [7].

The most recognized work of Christiane Nüsslein-Volhard, conducted together with Eric Wieschaus, followed the initial study with a much broader focus. The two researchers led a newly founded group in the European Molecular Biology Laboratory in Heidelberg, studying particularly the processes driving the patterning of *Drosophila melanogaster*. In their research, large scale genetic screens were conducted, with the basic aim of identifying genes that affected the outcomes of embryonic development processes. To generate a large amount of random mutations in the *drosophila* specimen, they were subjected to treatment with ethyl methanesulfonate. This compound is a known mutagen and teratogen, causing guanine alkylation based nucleotide substitution [8]. This compound, applied to the *drosophila* specimen, yielded a large amount of random mutants of different phenotypical characteristics. Embryos in various developmental stages were subjected to mutagenesis, which allowed for complex characterisation of the distinct steps of patterning. This resulted in characterisation of 15 loci, mutations in which significantly affect the pattern of larval segments. The genes were grouped, depending on the stage in which they exhibit their action. Three main groups were described: Gap, pair rule, and segment polarity genes. Gap gene expression arises in response to gradient of maternal-effect genes. These gradients result in specific position placement of particular gap genes, which indicate the initial pattern of segmentation [9]. Three genes from this group were identified by Nüsslein-Volhard and Wieschaus. *Krüppel*, *knirps* and *hunchback* were found to be expressed at the

early embryonic periods, acting as regulators of expression of further genes responsible for *drosophila* segmentation. Lack of their expression resulted in severe embryo deformation, usually associated with deletion of large continuous segment stretches [10]. The further steps of segment formation were described to be directed by pair rule genes. This group derives its name from their specific pattern of expression, which takes form of alternating bands that span across the length of the embryo [11]. These regions correspond to the location of so called parasegments, primordial segments containing anterior part of one final segment and posterior part of the next (more anterior) segment. The expression of pair rule genes is regulated by gap genes, as well as mutual interactions between the pair rule genes themselves. Impaired expression of any of those genes results in failure to develop their respective alternating parasegments [12]. The study of Nüsslein-Volhard and Wieschaus identified five pair rule genes: *even-skipped*, *hairy*, *odd-skipped*, *paired* and *runt*. The final class of genes responsible for *drosophila* segmentation are the segment polarity genes. As the name indicates, these genes function to establish the anteroposterior polarity of the final segments. Expressed in response to the communication between cells of adjacent parasegments, their effects cause the differentiation of posterior and anterior segment regions through the regulation and transmission of signals with the use of Wnt and Hedgehog signalling pathways. Lack of expression of certain segment polarity genes results in abnormal boundaries between the final segments, or failure in specific segment development [13]. Nüsslein-Volhard and Wieschaus identified six of those genes: *cubitus interruptus*, *wingless*, *gooseberry*, *hedgehog*, *fused* and *patch*. The results of this study were a major breakthrough in describing the processes driving early *drosophila* patterning. This knowledge, complemented by further research identifying the particular genes responsible for the mutations observed in the genetic screens, resulted in a complex “map” of *drosophila* embryogenesis. The study has become recognized around the world, not only because of the significance of the results for embryology and developmental biology, but also considering the enormous scale of the conducted research and the vast amount of work conducted to achieve the results. The research was significant enough to bring the two researchers a Noble Prize in Physiology and Medicine in 1995.

Implications and further *drosophila* applications

Christiane Nüsslein-Volhard later continued her work on the *drosophila* model, further investigating the genes identified in her breakthrough study. This research resulted in discovery and isolation of the *bicoid* protein, deposited in the anterior of the egg

by the female fruit fly, considered to be the first described morphogen [14,15]. Later, she focused on translating the knowledge gained in the research of *Drosophila* into vertebrate models. Working in the Max Planck Institute for Developmental Biology in Tübingen, she began study investigate fish as a vertebrate analogue of the fruit fly model. Her lab quickly expanded, soon housing a large amount of Zebrafish specimen. The results of the experiments were published in a form of a complex manual outlining the details of work with Zebrafish and their potential scientific applications [16,17]. The fish model is now widely used in the research on early embryonic development and patterning.

Her research has found significance not only as a relatively accurate description of early developmental processes of *Drosophila melanogaster*. Later studies found analogues of the described genes and proteins across other species, including humans. This caused the *Drosophila* to become a highly regarded tool for various types of research, serving as a point of reference for further vertebrate studies. Apart from being an excellent model for large scale genetic research, the fruit fly found further applications in other fields of modern research. Firstly, *Drosophila* has been used in the studies of regenerative biology and medicine for almost 40 years. These studies included the investigation of the regenerative capacity of the imaginal disc fragments transplanted to a different regions of the embryo in various developmental stages, as well as the analysis of basic molecular mechanisms of wound healing. The important results of such research include description of many factors and pathways regulating wound healing processes, and major contribution to the knowledge about regulation of stem cell activity [18]. Secondly, the fruit fly can also be used in the process of drug discovery, serving as an alternative to the initial stage of cell culture, with potential to mimic *in vivo* disease conditions through genetic background manipulation. While the studies still need to be validated in further research phases, this methods could serve as a complement to the initial investigation of the cellular drug response [19]. Finally, the modern bioengineering studies use *Drosophila* as a medium of cell and tissue manipulation investigation [20]. All of the above proves that *Drosophila melanogaster* was, is and will be an extremely important model for scientific research. The knowledge gained from that model improves the basic understanding of developmental processes, while the model itself is relatively easy to maintain, analyse and translate the results onto other species. While models such as Zebrafish present better with other vertebrates, *Drosophila* remains a very important element of genetic research, finding even more applications with the development of current science and medicine.

Ethical approval

The conducted research is not related to either human or animal use.

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Conflict of interest statement

The authors declare they have no conflict of interest.

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