Drosophila as a Model Organism

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Abstract

Drosophila melanogaster has been widely used in classical and modern genetics for more than 100 years. The history of the *Drosophila* model in the study of various aspects of life sciences will be summarized in this chapter. Furthermore, commonly used techniques and tools with *Drosophila* models will be briefly described, with a special emphasis on the advantages of *Drosophila* models in the study of various human diseases.

Keywords

Drosophila · History · Biology · Chromosome · Genome · GAL4-UAS

1.1 History of Studies with *Drosophila*

Drosophila melanogaster is one of the most commonly used experimental organisms and was first studied experimentally by Dr. Castle (Castle 1906) and used by Dr. Morgan for genetic experiments from 1909 (Sturtevant 1959). During the following 30 years, a number of the main principles of classical genetics were established by studying *Drosophila*, which advanced our understanding of genes, chromosomes, and the inheritance of genetic information (Ashburner and Bergman 2017). Mutagenesis techniques using radiation and chemicals were also developed with *Escherichia coli*, yeast, and *Drosophila*, allowing scientists to clarify gene functions by studying the phenotypes induced by mutations.

After 1970, various molecular, developmental, and biological techniques began to be applied to *Drosophila*, such as gene cloning, hybridization, P-element-based transformation, and clonal analyses. These techniques allowed scientists to perform analytical rather than descriptive studies on the development and behavior of *Drosophila*. In 1994, the Nobel Prize in Physiology or Medicine was awarded to Dr. Lewis for his studies with *Drosophila* to elucidate gene structures, as well as Drs. Weischaus and Nusslein-Volhard for their pioneering work on embryogenesis and the identification of a large number of genes involved in all aspects of *Drosophila* development, including segmentation. Most of the mammalian homologues of these genes were then found to be essential for mammalian development. In addition, many tumor suppressor genes were initially identified in *Drosophila*, and their human homologues were subsequently detected and proven to play important roles in oncogenesis.

The genome project of *Drosophila melanogaster* (*D. melanogaster*) was completed in 2000.

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A few years later, the human genome project was also finished, and comparisons of both genome sequences revealed high homologies between the *Drosophila* and human genomes, thereby confirming the importance of *Drosophila* as a model to study human diseases (Adams et al. 2000; Myers et al. 2000). Nearly 75% of human disease-related genes have been estimated to have functional orthologues in *Drosophila* (Pandy and Nichols 2011; Yamamoto et al. 2014). Overall identity at the nucleotide or amino acid sequence between *Drosophila* and mammals is approximately 40% between homologues. Regarding the conserved functional domains of proteins, identity may be more than 80%. The completion of the fly genome also promoted the study of transcription, protein binding to specific DNA sequences, and genetic variations at the molecular level. Based on genome information, we may perform RNA-sequence analyses or microarrays for expression profiling, targeted to all known or predicted coding regions or against the entire *Drosophila* genome including noncoding regions. We may also perform the genome-wide mapping of binding sites for chromatin-associated proteins at a high resolution using DNA adenine methyltransferase identification (DamID) (Sun et al. 2003; Bianchi-Frias et al. 2004), chromatin immunoprecipitation assays (ChIP assays), or a ChIP-sequence analysis (MacAlpine et al. 2004; Birch-Machin et al. 2005). Genome-wide surveys for polymorphisms using high-throughput PCR strategies are now also available (Glinka et al. 2003). Thus, *Drosophila* is always at the forefront of modern biology, in which genes, gene engineering, and other new findings are often achieved first in *Drosophila* and then generalized to other organisms including humans. It is important to note that the Nobel Prize in Physiology or Medicine in 2017 was awarded to Drs. Hall, Rosbash, and Young for their discovery of the molecular mechanisms controlling the circadian rhythm in *Drosophila*.

1.2 Biology of *Drosophila*

Drosophila has a relatively rapid life cycle. A single fertile mating pair may produce hundreds of genetically identical offspring in approximately 10 days at 25 °C. This is markedly faster than the commonly used rodent models. *Drosophila* may also be regarded as a model organism defined by its developmental stage: the embryo, larva, pupa, and adult (Pandy and Nichols 2011).

After fertilization, embryos undergo highly synchronized nuclear division cycles. These cycles, which are composed of only G and S phases, proceed very rapidly, requiring approximately 10 min for one cycle to form a multinuclear syncytial blastoderm. After nine nuclear division cycles, most nuclei move to the surface of the embryo and undergo four successive nuclear divisions at the surface of the embryo. These nuclei then simultaneously cellularize to form a cellular blastoderm. After cellularization, they undergo segmentation processes. Early embryos store large amounts of DNA replication enzymes that are enzymatically and cytologically characterized by the embryo (Yamaguchi et al. 1991). The embryo may be used in studies on fundamental developmental biology by examining pattern formation, cell fate determination, organogenesis, central/peripheral neuronal development, and axon pathfinding.

The larva, particularly the wandering third instar larva, is commonly used to study developmental and physiological processes as well as less complex behaviors such as foraging. A group of cells called imaginal discs produce the future adult external structures of *Drosophila* and are contained within the larva. They are primarily composed of an undifferentiated epithelium. In the late third instar larval stage and subsequent pupal stage, imaginal discs undergo morphological changes that produce adult external structures such as the antenna, compound eye, wings, and

legs. Imaginal disc cells undergo a typical G1, S, G2, and M phase cycle. In terms of cell cycle studies, eye imaginal discs in the third instar larvae are particularly useful. Cells in the anterior region to the morphogenetic furrow undergo random cell division, and those at the morphogenetic furrow are arrested at the G1 phase and then synchronously undergo the S, G2, and M phases to double the cell number. Then all cells fall into the G1/G0 and undergo differentiation. Thus, the eye imaginal disc provides a naturally occurring synchronized cell system that is very useful for characterizing the genes involved in the regulation of the cell cycle and DNA replication (Yamaguchi et al. 1999). The differentiation processes of eight (R1–R8) photoreceptor cells have also been studied in detail. Clarification of the mechanisms responsible for the developmental processes of imaginal discs has provided significant insights into *Drosophila* and human biologies. Furthermore, learning and memory assays are possible with larvae.

In the pupal stage, *Drosophila* undergoes metamorphosis, and during metamorphosis, imaginal discs undergo cell proliferation, differentiation, and organogenesis to produce various adult external structures, while most larval tissues undergo autophagy and cell death (Aguila et al. 2007). Cells undergo these processes in response to the hormone 20-hydroxyecdysone (ecdysone), which initiates larval-prepupal and prepupal-pupal transitions (Baehrecke 1996). Consequently, *Drosophila* undergoes morphological changes with the tight regulation of various biological pathways. During metamorphosis, the metabolic rate of *Drosophila* follows a U-shaped curve in which energy consumption is high during the first stages, declines toward the mid-pupal stage, and increases again toward the last phases of the larval-adult transformation (Merkey et al. 2011). Further details on the metabolic changes that occur during the development of *Drosophila* are described in Chap. 14.

The *Drosophila* adult provides a complex model organism that is somewhat similar to mammals in many aspects. The adult fly has organs that are functionally similar to the mammalian heart, lung, kidney, gut, and reproductive tract. The adult fly brain contains more than 100,000 neurons that form discrete circuits and neuropils, which mediate complex behaviors including wake and sleep circadian rhythms, learning and memory, feeding, aggression, courtship, and grooming. More significantly, the responses of *Drosophila* to various drugs that act on the central nervous system are similar to the effects observed in mammals (Rothenfluh and Heberlein 2002; Satta et al. 2003; Wolf and Heberlein 2003; Nichols 2006; Andretic et al. 2008). Therefore, *Drosophila* provides a useful model for screening therapeutic drugs for various human neuropathies.

1.3 Chromosomes of *Drosophila*

1.3.1 Overview

D. melanogaster has four sets of chromosomes, the X and Y sex chromosomes, two autosomal chromosomes 2 and 3, and the very small chromosome 4 (Metz 1914; Deng et al. 2007). Female flies carry two X chromosomes and males carry a single X and Y chromosome. Females and males carry two sets of the autosomal second, third, and fourth chromosomes. The X chromosome is acrocentric and may be divided into two arms by the centromere, a large left arm and a markedly smaller right arm. The Y chromosome is also acrocentric with a slightly longer long arm and shorter arm. In contrast, chromosomes 2 and 3 are metacentric with the centromere located in nearly the center of two left and right arms, named 2L, 2R, 3L, and 3R, respectively. The fourth chromosome is also acrocentric, carrying a small left arm and larger right arm.

Drosophila chromosomes may be functionally and structurally divided into heterochromatic and euchromatic regions. Heterochromatin is designated as the darkly staining regions in karyotyping. The heterochromatic region is also known to be late replicating in the S phase of the cell cycle and is enriched with highly repetitive nucleotide sequences and transposable elements (Dimitri 1997). The X, second, and third chromosomal regions adjacent to the centromeres are

darkly staining and are designated as pericentric heterochromatin. The Y and fourth chromosomes are also darkly staining and entirely heterochromatic, although the fourth chromosome has a small euchromatic right arm. The gene densities of the heterochromatic regions of the genome are lower than those of euchromatic regions. The Y chromosome is not necessary for the viability of *Drosophila*; however, XO males lacking the Y chromosome are sterile. Furthermore, XXY flies are female, indicating that the Y chromosome plays no role in sex determination in *Drosophila*. Sex is determined by the balance between the X chromosome and autosome in *Drosophila*: X:A = 1 is female and X:A = 0.5 is male.

1.3.2 Polytene Chromosomes

In *Drosophila*, after differentiation, most cells undergo endoreplication in which the S and G phases are repeated without any M phase. The most typical endoreplicating tissue in *Drosophila* is the larval salivary glands. In the case of the third instar larval salivary gland, the ploidy level reaches 1024 (Rodman 1967; Hammond and Laird 1985). The levels of polyploidy are mainly reached by the euchromatic regions of the genome because the heterochromatic regions are under-replicated. Furthermore, homologous chromosomes undergo somatic pairing in the polytene chromosome. Thus, the combination of polyploidy and pairing may produce 1024 DNA strands for each euchromatic chromosome arm. All chromosome arms corresponding to X, 2L, 2R, 3L, 3R, and the small 4 expand from a central region called the heterochromatic chromocenter (Fig. 1.1). The heterochromatic chromocenter is composed of pericentric heterochromatin and, in the case of males, the Y chromosome. Polytene chromosomes are sufficiently large to be easily observed using a standard light microscope. Each of the euchromatic arms shows a unique banding pattern caused by the differential condensation of chromatin to form darkly stained bands and less stained interbands (Fig. 1.1).

In *Drosophila*, the band pattern of salivary gland polytene chromosomes is highly reproducible among individuals (Bridges 1935). Each large arm is cytologically divided into 20 roughly equal numbered segments (X = 1–20; 2L = 21–40; 2R = 41–60; 3L = 61–80; 3R = 81–100; 4 = 101–102). Each of these num-

Fig. 1.1 In situ hybridization of the *white* gene on salivary gland polytene chromosomes. The arrowhead indicates the *white* gene locus (3B6)

bered segments is further divided into six roughly equal lettered segments, A to F, and the bands in each lettered segment are numbered. Therefore, each band has a unique address, and its position is easily discernible from the address. Moreover, the positions of genes may now be mapped on these addresses.

Various research tools have been developed to mark functional regions on polytene chromosomes. Anti-phosphorylated RNA polymerase II is used to mark the transcriptionally active domain of polytene chromosomes, and anti-heterochromatin protein 1 (HP-1) marks heterochromatic and heterochromatin-like regions on chromosomes (Kato et al. 2007, 2008). By immunostaining chromatin-binding proteins with specific antibodies in combination with various markers, it is possible to identify the protein of interest binding to the relevant functional region of chromosomes, such as euchromatic or heterochromatic and transcriptionally active or inactive regions.

1.3.3 Balancer Chromosomes

Balancer chromosomes are an extremely valuable tool in studies on *Drosophila*. They contain extensive inversions through the entire chromosome that prevent the recovery of chromosome exchange events, thereby isolating and maintaining the sequences in the balancer and balanced chromosome. They do not prevent crossing over but inhibit the recovery of exchanged chromatids (Kaufman 2017). Balancer chromosomes are used to stably maintain lethal and sterile mutations in the *Drosophila* stock without the selection process. Balancer chromosomes are also useful for effectively screening for mutations by maintaining the linear integrity of a mutagenized homologue (Kaufman 2017). These processes are very difficult to perform in other model organisms, such as the mouse without balancers.

Balancer chromosomes carry a recessive lethal mutation that is not related to the lesion being balanced and, thus, may efficiently balance lethal and sterile mutations. Balancer chromosomes also carry dominant visible mutations, and scientists may easily follow flies carrying the balancer in crossing schemes. Many balancer chromosomes also carry a set of recessive visible mutations that are useful for designing screens and distinguishing complex genotypes. Transgenic flies carrying a set of new and useful visible markers to the balancer have been developed as follows. Transgenes expressing visible markers, such as LacZ, GFP, or other fluorophores, in various spatial and temporal patterns have been inserted into different balancers as new and useful dominant markers. These transgenic flies may be used to easily distinguish the marked balancer flies from non-balancer flies at various developmental stages (Kaufman 2017). A list of balancers may be found at the BDSC site (http://flystocks.bio.indiana.edu/Browse/balancers/balancer_main.htm).

1.3.4 *Drosophila* Genome

The genome size of *D. melanogaster* is approximately 180 Mb, with 2/3 (120 Mb) representing the euchromatic region and 1/3 (60 Mb) the heterochromatic region. After the first report of the *D. melanogaster* genome by the consortium of the Berkley Drosophila Genome Project and Celera Genomics (Adams et al. 2000; Myers et al. 2000), the annotation of the genome has been revised several times by incorporating data from genome-wide RNA sequencing analyses and those on heterochromatin (modENCODE et al. 2010; Graveley et al. 2011; Boley et al. 2014; Brown et al. 2014; Chen et al. 2014; Kaufman 2017). Based on the current release, the total sequence length is 143,726,002 bp with a total gap length mainly in heterochromatin, including major and minor scaffolds of 1,152,978 bp (Kaufman 2017). The sequence is assembled into 1870 scaffolds with the majority of the sequence, 137.6 Mbp, residing on the seven chromosome arms (X, Y, 2L, 2R, 3L, 3R, and 4) and the entire mitochondrial genome. The sequence includes contiguous portions of the pericentric heterochromatin of X, 2, 3, and 4. Some may be mapped to the highly repetitive rRNA-encoding genes in the nucleolus organizer of X and Y (He et al. 2012).

Annotation of the genome currently identifies 17,726 genes, 13,907 of which are protein coding that encode 21,953 unique polypeptides. The remaining 3821 identified loci are genes encoding various types of noncoding RNA, 147 for rRNA, 313 for tRNA, 31 for snRNA, 288 for snoRNA, 256 for miRNA, 2470 for lncRNA, and 315 for pseudogenes (Kaufman 2017). The importance of many of these genes is now being recognized, particularly in relation to human diseases. Further details on noncoding RNA related to human diseases will be described in Chap. 8.

1.4 Strategies and Techniques to Study Human Diseases Using *Drosophila*

There are two main strategies to study human diseases using the *Drosophila* model: forward and reverse genetics.

1.4.1 Studies with Forward Genetics

In forward genetics, mutations are induced at random, and flies are screened for a phenotype of interest. Mutations may be generated by ethyl methanesulfonate (EMS) or the insertion of transposons, such as P-element and piggyback (Venken and Bellen 2014). Mutants may also be isolated by screen RNAi libraries or chromosome deficiency kits that cover 95% of the euchromatic region of the *Drosophila* genome (Ida et al. 2009; Cook et al. 2012). These strategies are useful for identifying uncharacterized mutations in already known disease-related genes as well as genes that have not yet been linked to disease. Therefore, this represents a useful strategy for identifying previously unknown genes and clarifying various biological events.

1.4.2 GAL4-UAS Targeted Expression System

A commonly used approach to express or knockdown specific genes in *Drosophila* is the so-called GAL4-UAS targeted expression system (Brand and Perrimon 1993). GAL4 is a yeast transcription factor that is used to control the spatial and temporal expression of target genes, which consequently directs gene activity at a specific developmental stage and specific cells and tissues. In one parental strain, promoter regions for a particular gene are designed to drive the expression of GAL4 in some tissues. In another strain, the GAL4-binding upstream-activating sequence (UAS) is placed in front of the transgene. When these two strains are genetically crossed, their progenies express the transgene in specific tissues driven by the GAL4-UAS system. In combination with RNA interference (RNAi), it is also possible to knockdown specific genes by expressing double-stranded RNAs targeted to specific mRNAs using the GAL4-UAS system. A useful resource for this purpose is the collection of UAS-RNAi responder strains of the Vienna Drosophila Stock Center (VDRC) (http://stockcenter.vdrc.at/control/main). These RNAi knockdown strains cover nearly 90% of all *Drosophila* protein-coding genes and are available to the research community from VDRC (Dietzl et al. 2007). The basic GAL4-UAS targeted expression system has been modified to further refine cell and tissue specificities as well as temporal expression specificities (Roman et al. 2001: McGuire et al. 2004).

1.4.3 Studies with Reverse Genetics

In reverse genetics, mutations are generated in *Drosophila* homologues of human genes to characterize their phenotypes in vivo. There are several approaches to knockdown or knockout genes in *Drosophila*. One strategy is targeted gene disruption using clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) (Beumer and Carroll 2014), transposon-mediated mutagenesis, and the excision of pre-existing transposable elements. The other is gene silencing with RNAi combined with the GAL4-UAS system or CRISPR (Mohr 2014). In addition to these loss-of-function studies, a wild-type or mutant form of a human disease-causing gene

may be introduced and overexpressed in *Drosophila* in order to examine its effects in specific tissues and organs (Feany and Bender 2000).

Drosophila is also useful for studying the pathogenesis of rare variants that are linked to human diseases (Ugur et al. 2016). There are US-based initiatives to identify human disease-causing genes by deep sequencing of the whole exomes or genomes of patients and their families, which are coordinated by the Centers for Mendelian Genomics (http://www.mendelian.org/) and Undiagnosed Diseases Network (UDN; http://undisgnosed.hms.harvard.edu/). Similar strategies have been performed in other countries, such as the UK (http://www.uk10k.org/) and China (Guangzhou Drosophila Resource Center and the Center for Genomic Sciences in the University of Hong Kong). These strategies are sometimes not sufficient for identifying the disease-causing gene if only a few individuals are assessed. In these cases, the *Drosophila* orthologue may be knocked out or down in order to examine phenotypes. If the observed phenotype is rescued by the expression of wild-type UAS-human-cDNA, but not by its human variant, disease causality may be confirmed (Bellen and Yamamoto 2015; Wangler et al. 2015).

1.5 Advantages of *Drosophila* in the Study of Human Diseases

Several model organisms are intensively studied in life sciences, such as the mouse, zebrafish, *Xenopus*, *Arabidopsis*, *Drosophila*, *Caenorhabditis elegans*, yeast, and *E. coli*. These are specific species that are extensively examined in research laboratories. Studies using these model organisms will advance our understanding of cellular functions, development, and human diseases. The knowledge obtained from these model organisms may also be applied to other organisms, which will result in the generalization of findings.

The following characteristics of *Drosophila* demonstrate that it is a good model organism. It is small, easy to handle, and inexpensive to maintain and manipulate in the laboratory. *Drosophila* has a short life span and produces a large number of offspring, which facilitates statistical analyses of the data obtained. *Drosophila* development is external, and, thus, it is very easy to follow using various microscopes. Many mutants and transgenic fly lines may be obtained from stock centers such as the Bloomington Drosophila Stock Center (https://bdsc.indiana.edu/), Kyoto Stock Center (http://www.dgrc.kit.ac.jp/), and VDRC. A plethora of information from previous experiments and discoveries is available. Sequencing of the genome is nearly complete, as described above. Homologues for nearly 75% of human disease-related genes have been identified (Pandy and Nichols 2011; Yamamoto et al. 2014). *Drosophila* shows complex behaviors including social activity. In addition, there are fewer ethical concerns because the insect is outside animal laws in many countries. In combination with genome-wide genetic screening, genome-wide analyses with deep sequencers, such as RNA-seq and ChIP-seq, and metabolomics analyses, *Drosophila* is now commonly used as a model to study human diseases with the aim of identifying novel biomarkers or therapeutic targets for human diseases together with the screening of candidate substances for their treatment (Pandy and Nichols 2011). *Drosophila* is now used in the study of various human diseases related to the central and peripheral nervous systems such as neurodegeneration, Alzheimer's disease (Chap. 3), Parkinson's disease (Chap. 4), triplet repeat expansion disease (Chap. 5), sleep disorders (Pandy and Nichols 2011), seizure disorders (Pandy and Nichols 2011), cognitive and psychosis disorders (Pandy and Nichols 2011), amyotrophic lateral sclerosis (Chap. 6), and Charcot-Marie-Tooth disease (Chap. 7). It is also used as a cancer model including tumor formation and metastasis (Chaps. 10 and 11). *Drosophila* may also be employed in the study of cardiovascular diseases (Pandy and Nichols 2011; Ugur et al. 2016). Although the fly heart has only one cardiac chamber, it may still be used to study some steps of heart development and its

defects. *Drosophila* also provides a model for inflammation/infectious diseases, metabolic disorders, and diabetes (Chaps. 13 and 14).

There are some limitations to *Drosophila* models. *Drosophila* does not possess hemoglobin (Adams et al. 2000; Myers et al. 2000), and, thus, *Drosophila* models cannot be generated for human diseases related to hemoglobin. Smaller organisms, such as yeast and *E. coli*, which have shorter generation times, smaller genomes, and produce more offspring than *Drosophila*, are preferred for the study of cell autonomous functions, such as DNA replication and repair. Therefore, an ideal study of human diseases will be a parallel analysis with relevant models. For example, cell autonomous effects will be studied in yeast, while multicellular or inductive events mediated by genes will be examined using *Drosophila.* A more accurate disease model needs to be established in the mouse. In any case, the benefits of *Drosophil*a may be summarized as follows. A number of genes related to human diseases have already been discovered and various useful techniques developed. Powerful tools for studying developmental/neurological disorders and cancer are now available. Therefore, *Drosophila* is a very effective model with more simplicity than mammalian models and greater complexity than yeast and bacterial models.

1.6 Commonly Used Websites for *Drosophila* Studies

Commonly used online databases for *Drosophila* studies are now available to support experimental design, the identification of relevant fly stocks, research tools, reagents such as antibodies, and related human diseases (Table 1.1). These databases are particularly useful for beginners. More

Table 1.1 A list of websites providing information about *Drosophila* or human diseases

Website	URL
FlyBase	http://flybase.org
modENCODE	http://modencode.sciencemag.org/drosophila/introduction
Drosophila Genomics Resource Center	https://dgrc.bio.indiana.edu/Home
Berkeley Drosophila Genome Project	http://www.fruitfly.org/
Drosophila Genomics and Genetic Resources	http://www.dgrc.kit.ac.jp/
Bloomington Drosophila Stock Center	https://bdsc.indiana.edu/
Vienna Drosophila Resource Center	http://stockcenter.vdrc.at/control/main
NIG-FLY	https://shigen.nig.ac.jp/fly/nigfly/
The Exelixis Collection at Harvard Medical School	https://drosophila.med.harvard.edu/
DRSC/TRiP Functional Genomics Resources	https://fgr.hms.harvard.edu/fly-in-vivo-rnai
FlyORF	http://flyorf.ch/index.php/orf-collection
FlyExpress 7	http://www.flyexpress.net/
FlyBook	http://www.genetics.org/content/flybook
FlyMove	http://flymove.uni-muenster.de/
Fly-FISH	http://fly-fish.ccbr.utoronto.ca/
Flygut	http://flygut.epfl.ch/
FlyMine	http://www.flymine.org/
Gene Disruption Project	http://flypush.imgen.bcm.tmc.edu/pscreen/index.php
The Interactive Fly	http://www.sdbonline.org/sites/fly/aimain/1aahome.htm
Textpresso for Fly	http://www.textpresso.org/fly/
BruinFly	http://www.bruinfly.ucla.edu/index.php
Virtual Fly Brain	https://www.virtualflybrain.org/site/vfb_site/home.htm
Fruit Fly Brain Observatory	http://fruitflybrain.org/
DroID – The Drosophila Interactions Database	http://flygut.epfl.ch/
J-FLY	http://jfly.iam.u-tokyo.ac.jp/index.html
Neuromuscular Disease Center	http://neuromuscular.wustl.edu/

specialized bioinformatics resources for *Drosophila* scientists are described in Chap. 15.

References

Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF, et al. The genome sequence of *Drosophila melanogaster*. Science. 2000;287:2185–95.

Aguila JR, Suszko J, Gibbs AG, Hoshizaki DK. The role of larval fat cells in adult *Drosophila melanogaster*. J Exp Biol. 2007;210:956–63.

Andretic R, Kim YC, Jones FS, Han KA, Greenspan RJ. *Drosophila* D1 dopamine receptor mediates caffeine-induced arousal. Proc Natl Acad Sci U S A. 2008;105:20392–7.

Ashburner M, Bergman CM. *Drosophila melanogaster*: a case study of a model genomic sequence and its consequence. Genome Res. 2017;15:1661–7.

Baehrecke EH. Ecdysone signaling cascade and regulation of *Drosophila* metamorphosis. Arch Insect Biochem Physiol. 1996;33:231–44.

Bellen HJ, Yamamoto S. Morgan's legacy: fruit flies and the functional annotation of conserved genes. Cell. 2015;163:12–4.

Beumer KJ, Carroll D. Targeted genome engineering techniques in *Drosophila*. Methods. 2014;68:29–37.

Bianchi-Frias D, Orian A, Delrow JJ, Vazquez J, Rosales-Nieves AE, Parkhurst SM. Hairy transcriptional repression targets and cofactor recruitment in *Drosophila*. PLoS Biol. 2004;2:E178.

Birch-Machin I, Gao S, Huen D, McGirr R, White RA, Russell S. Genomic analysis of heat-shock factor targets in *Drosophila*. Genome Biol. 2005;6:R63.

Boley N, Stoiber MH, Booth BW, Wan KH, Hoskins RA, et al. Genome-guided transcript assembly by integrative analysis of RNA sequence data. Nat Biotechnol. 2014;32:341–6.

Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development. 1993;118:401–15.

Bridges CB. Salivary chromosome maps with a key to the banding of the chromosomes of *Drosophila melanogaster*. J Hered. 1935;26:60–4.

Brown JB, Boley N, Eisman R, May GE, Stoiber MH, et al. Diversity and dynamics of the *Drosophila* transcriptome. Nature. 2014;512:393–9.

Castle WE. Inbreeding, cross-breeding and sterility in *Drosophila*. Science. 1906;23:153.

Chen ZX, Sturgill D, Qu J, Jiang H, Park S, et al. Comparative validation of the *D. melanogaster* modENCODE transcriptome annotation. Genome Res. 2014;24:1209–23.

Cook RK, Christensen SJ, Deal JA, Coburn RA, Deal ME, Gresens JM, Kaufman TC, Cook KR. The generation of chromosomal deletions to provide extensive coverage and subdivision of the *Drosophila melanogaster* genome. Genome Biol. 2012;13:R21.

Deng Q, Zeng Q, Qian Y, Li C, Yang Y. Research on the karyotype and evolution of *Drosophila melanogaster* species group. J Genet Genomics. 2007;34:196–213.

Dietzl G, Chen D, Schnorrer F, Su KC, Barinova Y, Fellner M, Gasser B, Kinsey K, Oppel S, Scheiblauer S, et al. A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature. 2007;448:151–6.

Dimitri P. Constitutive heterochromatin and transposable elements in *Drosophila melanogaster*. Genetica. 1997;100:85–93.

Feany MB, Bender WW. A *Drosophila* model of Parkinson's disease. Nature. 2000;404:394–8.

Glinka S, Ometto L, Mousset S, Stephan W, De Lorenzo D. Demography and natural selection have shaped genetic variation in *Drosophila melanogaster:* a multilocus approach. Genetics. 2003;165:1269–78.

Graveley BR, Brooks AN, Carlson JW, Duff MO, Landolin JM, et al. The developmental transcriptome of *Drosophila melanogaster*. Nature. 2011;471:473–9.

Hammond MP, Laird CD. Control of DNA replication and spatial distribution of defined DNA sequences in salivary gland cells of *Drosophila melanogaster*. Chromosoma. 1985;91:279–86.

He B, Caudy A, Parsons L, Rosebrock A, Pane A, et al. Mapping the pericentric heterochromatin by comparative genomic hybridization analysis and chromosome deletions in *Drosophila melanogaster*. Genome Res. 2012;22:2507–19.

Ida H, Suzusho N, Suyari O, Yoshida H, Ohno K, Hirose F, Itoh M, Yamaguchi M. Genetic screening for modifiers of the DREF pathway in *Drosophila melanogaster*: identification and characterization of HP6 as a novel target of DREF. Nucleic Acids Res. 2009;37:1423–37.

Kato M, Kato Y, Nishida M, Hayakawa T, Haraguchi T, Hiraoka Y, Inoue YH, Yamaguchi M. Functional domain analysis of human HP1 isoforms in *Drosophila*. Cell Struct Funct. 2007;32:57–67.

Kato Y, Kato M, Tachibana M, Shinkai Y, Yamaguchi M. Characterization of *Drosophila* G9a *in vivo* and identification of genetic interactants. Genes Cells. 2008;13:703–22.

Kaufman TC. A short history and description of *Drosophila melanogaster* classical genetics: chromosome aberrations, forward genetic screens, and the nature of mutations. Genetics. 2017;206:665–89.

MacAlpine DM, Rodriguez HK, Bell SP. Coordination of replication and transcription along a *Drosophila* chromosome. Genes Dev. 2004;18:3094–105.

McGuire SE, Roman G, Davis RL. Gene expression systems in *Drosophila*: a synthesis of time and space. Trends Genet. 2004;20:384–91.

Merkey AB, Wong CK, Hoshizaki DK, Gibbs AG. Energetics of metamorphosis in *Drosophila melanogaster*. J Insect Physiol. 2011;57:1437–45.

Metz CW. Chromosome studies in the Diptera. J Exp Zool. 1914;17:45–59.

modENCODE Consortium RS, Ernst J, Kharchenko PV, Kheradpour P, et al. Identification of functional elements and regulatory circuits by *Drosophila* modENCODE. Science. 2010;330:1787–97.

Mohr SE. RNAi screening in *Drosophila* cells and in vivo. Methods. 2014;68:82–8.

Myers EW, Sutton GG, Delcher AL, Dew IM, Fasulo DP, et al. A whole-genome assembly of *Drosophila*. Science. 2000;287:2196–204.

Nichols CD. *Drosophila melanogaster* neurobiology, neuropharmacology, and how the fly can inform central nervous system drug discovery. Pharmacol Ther. 2006;112:677–700.

Pandy UB, Nichols CD. Human disease models in *Drosophila melanogaster* and its role of the fly in therapeutic drug discovery. Pharmac Rev. 2011;63:411–36.

Rodman TC. DNA replication in salivary gland nuclei of *Drosophila melanogaster* at successive larval and prepupal stages. Genetics. 1967;55:375–86.

Roman G, Endo K, Zong L, Davis RL. P[switch], a system for spatial and temporal control of gene expression in *Drosophila melanogaster*. Proc Natl Acad Sci U S A. 2001;98:12602–7.

Rothenfluh A, Heberlein U. Drugs, flies, and videotape: the effects of ethanol and cocaine on *Drosophila* locomotion. Curr Opin Neurobiol. 2002;12:639–45.

Satta R, Dimitrijevic N, Manev H. *Drosophila* metabolize 1,4-butanediol into gamma-hydroxybutyric acid in vivo. Eur J Pharmacol. 2003;473:149–52.

Sturtevant AH. Thomas Hunt Morgan. Biogr Mem Natl Acad Sci. 1959;33:283–325.

Sun LV, Chen L, Greil F, Negre N, Li TR, Cavalli G, Zhao H, Van Steensel B, White KP. Protein–DNA interaction mapping using genomic tiling path microarrays in *Drosophila*. Proc Natl Acad Sci. 2003;100:9428–33.

Ugur B, Chen K, Bellen HJ. *Drosophila* tools and assays for the study of human diseases. Dis Models Mech. 2016;9:235–44.

Venken KJ, Bellen HJ. Chemical mutagens, transposons, and transgenes to interrogate gene function in *Drosophila melanogaster*. Methods. 2014;68:15–28.

Wangler MF, Yamamoto S, Bellen HJ. Fruit flies in biomedical research. Genetics. 2015;199:639–53.

Wolf FW, Heberlein U. Invertebrate models of drug abuse. J Neurobiol. 2003;54:161–78.

Yamaguchi M, Date T, Matsukage A. Distribution of PCNA in *Drosophila* embryo during nuclear division cycles. J Cell Sci. 1991;100:729–33.

Yamaguchi M, Hirose F, Inoue YH, Shiraki M, Hayashi Y, Nishi Y, Matsukage A. Ectopic expression of human p53 inhibits entry into S-phase and induces apoptosis in the *Drosophila* eye imaginal disc. Oncogene. 1999;18:6767–75.

Yamamoto S, Jaiswal M, Charng W-L, Gambin T, Karaca E, Mirzaa G, Wiszniewski W, Sandoval H, Haelterman NA, Xiong B, et al. A *Drosophila* genetic resource of mutants to study mechanisms underlying human genetic diseases. Cell. 2014;159:200–14.