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Fruit fly as a model organism in the study of human diseases and drug discovery

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Drug discovery is a dynamic yet an ever-lasting topic in medicine that heavily relies on the application of model organisms. Through the use of suitable model organisms, pre-clinical testing of new drug candidates can be carried out extensively prior to further testing with humans. This has been proven, so far, as one of efficient ways to limit the emergence of new substances that are potentially harmful to individuals. Nevertheless, increasing public interest in the ethical issues raised by the use of live model organisms, such as mice and rats, pushes urgent needs for alternative model organisms. To this end, fruit fly *Drosophila melanogaster* might be an appropriate model organism to be accounted for. With its long-standing history of use, *Drosophila* is an insect behind the revelation of striking similarity to humans as to basic biological mechanisms and their tight controls to maintain homeostasis. Impairment of such events in *Drosophila* is linked to the emergence of metabolic-related diseases such as obesity and diabetes mellitus, the unsolved cases of neurodegenerative diseases, and the fall of host immune responses against various infectious agents. With a high genetic similarity, about 75%, to humans, ease of maintenance, and the availability of various disease models constructed through genetic manipulation and/or chemical induction, *Drosophila* has been a very promising model organism in the field of drug discovery. With this in mind, it would be not surprising if this tiny yet powerful model organism will soon substitute our current *in vivo* platform in the pre-clinical testing of new drug candidates.

INTRODUCTION

For the last decade, the emergence of sophisticated research tools, techniques, and model organisms in the field of medical and pharmaceutical sciences has extraordinarily advanced our understanding on the pathogenesis of diseases and their mechanisms at cellular and molecular levels that leads to the discovery of new effective drugs. In drug discovery research, the use of model organisms as an *in vivo* platform at a pre-clinical level for testing new drug compounds is still a commonly used method (Breyer *et al*. 2015; McGonigle & Ruggeri, 2014; Ruggeri *et al*. 2014). Through the use of various model organisms, careful investigations regarding the pathophysiology of diseases along with pharmacologic approaches that can be used to treat these diseases can be achieved in a proper manner (Breyer *et al*. 2015; McGonigle & Ruggeri, 2014). In addition, model organisms are also very useful to provide a general description on the possible mechanism of action(s) of drug candidates along with their potential toxicities that may occur before they are clinically tested in humans (McGonigle & Ruggeri, 2014; Vogel & Vogel, 2013).

Some animals commonly used in the pre-clinical testing of new drug candidates are mice, rats, guinea pigs, rabbits, cats, and dogs (Vogel & Vogel, 2013; Zuberi & Lutz, 2016). Phylogenetically, these animals have a close kinship with humans and therefore can provide sufficient and accurate information about the pathogenesis of diseases at the cellular and molecular levels (Vogel & Vogel, 2013). However, increasing concern on the animal welfare and awareness of the concept of animal rights has begun to limit the use of these animals in a pre-clinical research (Giacomotto & Ségalat, 2010; Pandey & Nichols,

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2011). This provides considerable pressure on researchers to immediately search for alternative model organisms that can be used in drug discovery research.

Several model organisms have been introduced as alternative *in vivo* platforms in the investigation of disease pathogenesis. One of the well-known model organisms is fruit fly *Drosophila melanogaster*. This organism has already been widely used to generate disease models for several types of human diseases and to test new drug candidates (Fernández‐Hernández *et al*. 2016; Pandey & Nichols, 2011; Strange, 2016; Ugur *et al*. 2016). *Drosophila* enables researchers to complete screening of drug candidates throughout by whole-animal experiments, a process difficult using traditional animal models such as mice, rats, and rabbits (Giacomotto & Ségalat, 2010; Pandey & Nichols, 2011; Ugur *et al*. 2016).

In this article, we will discuss the use of *Drosophila* as a model organism in drug discovery research. The purpose of such discussion is to provide researchers with inspiration with which they think to start using *Drosophila* in their study on the pathogenesis of human diseases including neurodegenerative diseases, cancer, cardiovascular disorders, infectious diseases, and disorders of metabolic syndrome such as obesity and diabetes mellitus. In addition to the above, the promising application of *D. melanogaster* in the discovery of new drugs will be briefly discussed. Furthermore, based on one author's (F. N.) experience in pioneering research using *Drosophila* at the Faculty of Pharmacy, Hasanuddin University (Indonesia), this model organism has great potential to be used by researchers in Indonesia because *Drosophila* can be kept for years using low-budget maintenance facilities which would not burden researchers in developing countries such as Indonesia.

WHY FRUIT FLY *Drosophila melanogaster*?

Fruit fly, also commonly known as vinegar fly, is an insect species in the Diptera order and the Drosophilidae family. This fly became the center of attention after Thomas Hunt Morgan introduced its use as a model organism in genetic research early 1900s (Markow, 2015). Until now *Drosophila* has been widely applied to explain various important biological phenomena that are also seen in humans, ranging from the role of apoptosis in development and immunity (Meier *et al*. 2000; Nainu *et al*. 2015; 2017; Nonaka *et al*. 2017), the effect of nutrition in regulating biological functions and individual age (Rajan & Perrimon, 2013), to the meaning of genetic defects against phenotypic disorders in organisms (Mackay, 2010; Nakanishi *et al*. 2011; Pandey & Nichols, 2011).

Drosophila is an invertebrate animal with a body size of about 3 mm (Panchal & Tiwari, 2017). The genome of the Drosophilidae family of insects is about 180 MB (megabases) that is divided into four chromosomes (Adams *et al*. 2000). With a small number of chromosomes, *Drosophila* becomes a preferred organism for the study on the mechanism of gene arrangement in a chromosome, regulation of gene activities and functions, and the pattern of mutations in eukaryotic organisms (Pandey & Nichols, 2011; Ugur *et al*. 2016; Wangler *et al*. 2015). Despite its simplicity, *Drosophila* genome is about 75% similar to human genome (Chien *et al*. 2002; Pandey & Nichols, 2011; Reiter *et al*. 2001). These are the bases for the potential use of *Drosophila* as a model organism suitable for research on disease mechanisms and drug discoveries.

Experimentally, fruit fly has several advantages. First, fruit fly is very easy to maintain requiring relatively low costs compared to other model organisms including zebrafish, mice, and rats (Giacomotto & Ségalat, 2010; Pandey & Nichols, 2011; Strange, 2016). This point is very beneficial to researchers having a limited size of research funds. Second, a female fly can produce 30-50 eggs every day, and each egg develops into an adult fly within 10 days. This is very different from mice that only produce a small number of offspring in 3-4 months (Panchal & Tiwari, 2017). Thus, the use of *Drosophila* facilitates reliable experimental results using large testing populations in a short period of time. Third, *Drosophila* has a short lifespan, 2-3 months. This is great advantage for studies of several biological processes such as the mechanism of aging (Brandt & Vilcinskas, 2013; He & Jasper, 2014; Sun *et al*. 2013). Finally, *Drosophila* can used in research without the requirement of ethical clearance (Panchal & Tiwari, 2017; Pandey & Nichols, 2011).

With a short lifespan of a few months, *Drosophila* lives shorter than mice, rats, rabbits, or humans. However, *Drosophila* experiences various phases of life as commonly found in other animals such as the embryonic phase, the juvenile phase (larvae), and the adult phase through a process called metamorphosis (Markow, 2015; Reaume & Sokolowski, 2006). Obviously, each develop-

mental phase lasts only a short period of time: embryos develop into first-instar larvae only in one day, and second-instar and third-instar larvae emerge in one and two days, respectively. And in the next 5 days, third-instar larvae develop into pupae, and adult flies eclose from pupal case (Reaume & Sokolowski, 2006).

Drosophila is a pioneer model organism in the identification of genes related to biological functions whose counterparts are also important in eukaryotic organisms, including humans. Examples of such genes include homeobox genes that play a crucial role in the control of entire developmental processes (Carroll, 1995; Pearson *et al*. 2005), a gene called dnc that was the first to be found important in learning processes (Dubnau & Tully, 1998), *period* whose identification has led to the understanding of circadian rhythm (Konopka & Benzer, 1971), and Tl has brought us the mechanisms of innate immunity (Lemaitre *et al*. 1996; Medzhitov *et al*. 1997). To date, eight Nobel Prizes in Physiology or Medicine were given to researchers who work with this tiny insect (Patel & Prokop, 2017).

Drosophila DATABASES AND STOCK CENTERS

In many studies, especially those related to the discovery and mechanisms of action of new drugs, the use of model organisms having mutation and transgenic genotypes is critically important (Bolon, 2004; Brad & Elizabeth, 2002; Snaith & Törnell, 2002). 'Mutant' is a term to describe the altered status of genetic materials in test animals while the term 'transgenic' means the process of increasing or decreasing the expression level of the existing genetic information. In the case of *Drosophila*, various types of mutants and transgenic flies can be generated easily owing to the availability of entire genetic manipulations (Adams *et al*. 2000; Hales *et al*. 2015; Li & Garza, 2004; Pandey & Nichols, 2011; Venken & Bellen, 2007). Until now, *Drosophila* lines having alterations on almost all individual genes have been produced and are widely used in research (Venken & Bellen, 2007; Yamamoto *et al*. 2014).

Drosophila is a model organism that has been successfully used for years in the screening of genes interested using either forward or reverse genetic methods (Hales *et al*. 2015). Forward genetics is a method to identify genes that are responsible for the emergence of certain phenotypes of researcher's interest (Gibson & Muse, 2009). For example, researchers may identify a gene responsible for converting the color of *Drosophila*'s eyes from red (mutant) to white (normal) using forward genetics. Conversely, reverse genetics is used to analyze phenotypes that emerge in model animals after altering genes of researcher's interest (Gibson & Muse, 2009). For example, researchers may find the roles of certain genes by looking at emerging phenotypes after introducing mutations on them.

The most important point in using *Drosophila* is to select appropriate lines with distinct genotypes. This is made possible through the use of organizations that distribute various types of *Drosophila* lines at low costs (Wangler & Bellen, 2017). For example, there are several wild-type strains from which researchers in the field of drug discovery may choose a strain most suitable for their contexts. Researchers can directly contact relevant stock centers by telephone or emails. In addition to such stock centers, there are researcher-based organizations, such as Drososhare in Europe, where dedicated researchers are in charge of distributing *Drosophila* lines to other researchers all over the world. The delivery of *Drosophila* can be done using ordinary postal packages, registered postal packages, or special postal packages with a customized delivery period.

Drosophila AS A SUITABLE ORGANISM FOR HUMAN DISEASE MODEL

To facilitate research on disease pathophysiology and drug discovery, the availability of human disease models is of a great help. Although the size and appearance of *Drosophila* are quite different from those of humans, this insect has been frequently used in various types of research to study the pathogenesis of human diseases (Pandey & Nichols, 2011; Reiter, 2005; Ugur *et al*. 2016). The use of *Drosophila* has provided important knowledge regarding the pathogenesis of various human diseases at cellular and molecular levels (Wangler & Bellen, 2017; Wangler *et al*. 2015). Accumulating information from such research is certainly very valuable in finding and tracing the working mechanism of new drug candidates.

1. *Drosophila* as a model organism to study cancer

Drosophila has made great contribution to studies on

the mechanism of the emergence of cancer and ways that can be used to overcome it (Gonzalez, 2013; Sonoshita & Cagan, 2017). A large number of genes and signaling pathways are involved in the creation and maintenance of our body. Any mistakes in gene expression and signal pathways could cause abnormal growth of cells called cancer, an event that was first discovered and characterized in *Drosophila* (Gonzalez, 2013; Sonoshita & Cagan, 2017). Examples of this kind are signaling pathways called Hedgehog and Hippo. Tumor develops in *Drosophila* when these pathways are impaired. Studies with *Drosophila* cancer models until now have shown that the process of cancer development in *Drosophila* and humans bear a striking resemblance to each other (Brumby & Richardson, 2005; Gonzalez, 2013; Rieder & Larschan, 2014; Wangler *et al*. 2015). In general, cancer in humans results from the uncontrolled growth of epithelial cells (Christofori & Semb, 1999). Using *Drosophila*, researchers have studied epithelial cell-derived cancer (Brumby & Richardson, 2005; Pandey & Nichols, 2011). There are four main hallmarks of cancer: uncontrolled cell division, resistance to cell growth-inhibiting signals, resistance to apoptosis-inducing signals, and migration and colonization at various places, referred as metastasis. All these hallmarks can be studied using *Drosophila* (Brumby & Richardson, 2005; Christofi & Apidianakis, 2013; Gonzalez, 2013). Most oncogenes and tumor suppressor genes responsible for the control of cancer in humans possess counterparts in *Drosophila*. The signaling pathway involving the oncogene product Ras was found for the first time in the visual system of *Drosophila* (Olivier *et al*. 1993; Simon *et al*. 1991), and this pathway plays an important role in the development of cancer in humans (Bier, 2005; Shaw & Cantley, 2006). Mutations in tumor suppressor genes *scribble, disc large 1*, and *lethal (2) giant larvae* in *Drosophila* induce cancer with all markers seen in humans, including metastatic processes metastasis (Brumby & Richardson, 2005).

The compound eye of *Drosophila* is often used as tissues to induce cancer (Miles *et al*. 2011; Rudrapatna *et al*. 2012). *Drosophila* eyes are made up of about 800 hexagon ommatidia, called facets, with "smooth" surface structures (Kumar, 2012). When cancer is induced, the surface of eyes is not smooth anymore and called "rough" eyes. This easy-to-find phenotype is widely used in the study of cancer pathogenesis as well as to examine the effectiveness of various treatments (Miles *et al*. 2011; Pandey & Nichols, 2011; Rudrapatna *et al*. 2012). Also, a test for survival of larvae and pupae is often taken to monitor the severity of cancer (Pandey & Nichols, 2011; Willoughby *et al*. 2013). In such studies, larvae bearing cancer, which is labeled with GFP, are placed in a 96-well plate, treated with drug candidates, and maintained for certain periods of time. The effectiveness of drug candidates is determined in two parameters: the number of survived larvae and the intensity of GFP fluorescence: GFP intensity is proportional to the rate of cancer growth (Pandey & Nichols, 2011; Reiter, 2005; Willoughby *et al*. 2013). Although *Drosophila* seems to be an ideal model organism for the study of cancer, there is a limitation: not all types of cancer found in humans can develop in *Drosophila* such as prostate cancer and breast cancer (Pandey & Nichols, 2011).

2. *Drosophila* as a model of neurodegenerative diseases

Since its introduction in the early 20th century by Thomas Hunt Morgan, *Drosophila* has been one of the favorite model organisms in a study on the anatomy and physiology of the nervous system in eukaryotes, including mammals (Bellen *et al*. 2010; Hales *et al*. 2015; Reiter, 2005). For example, mutations in a gene coding for Notch were identified in 1915 and reported a year later as a mutation that causes wing malformations in *Drosophila* (Bellen *et al*. 2010). This discovery paved the way for subsequent discoveries, including Delta as a Notch ligand. Eventually, the Notch signaling pathway in *Drosophila* and similar pathways in vertebrates, including humans, have been shown to play an important role in neural activities (Bellen *et al*. 2010).

An advantage in using fruit fly is that neurons can be completely removed without killing animals. In addition, any human genes in question can be expressed at certain time points and certain tissues (Pandey & Nichols, 2011; Reiter, 2005). Furthermore, the detection of gene products, i.e., proteins and mRNAs, in *Drosophila* is easier than in mice and rats. The expression patterns of gene interested can be determined by *in situ* detection of mRNA and monitoring tags fused to cognate proteins. Finally, *Drosophila* is a relatively short-living and thus suitable to monitor the pathogenesis of neurodegenerative diseases (Pandey & Nichols, 2011).

Using *Drosophila* researchers can conduct both gain-of-function and loss-of-function experiments more easily than using other model animals. Once *Drosophila* lines showing interesting phenotypes related neuronal actions, researchers carry out 'rescue' experiments by ectopically expressing candidate genes, even human genes, in a specific spatio-temporal manner. Also, researchers can screen a library of *Drosophila* lines that have lost candidate genes to find lines showing phenotypes of interest. However, it should be noted that not all *Drosophila* mutants survive until adulthood, and that phenotypes produced by mutating interesting genes in *Drosophila* might not those expected from human diseases (Reiter, 2005).

Drosophila has been used extensively to study biochemical and genetic processes that occur in eukaryotic nervous system (Bellen *et al*. 2010; Hales *et al*. 2015; Wangler *et al*. 2015). Particularly, *Drosophila* is now extensively used to investigate neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease, epilepsy, and amyotrophic lateral sclerosis (Pandey & Nichols, 2011; Reiter, 2005; Wangler & Bellen, 2017; Yamamoto *et al*. 2014). From such research, we have so far learned that most neurodegenerative diseases caused by the progressive loss of specific neurons are closely related to the formation of toxic protein aggregates in intracellular environments (Pandey & Nichols, 2011; Reiter, 2005). Also, as known for humans, the emergence of neurodegenerative diseases in *Drosophila* becomes frequent as animals age (Bonner & Boulianne, 2011).

3. *Drosophila* as a model to study infectious diseases

Like most other insects, *Drosophila* is only equipped with the innate immune system (Hoffmann, 2003) of which structure is, unexpectedly, quite similar to humans' (Buchon *et al*. 2014; Hoffmann, 2003). Therefore, *Drosophila* has been used to investigate the basic mechanisms of the innate immune system of humans. In addition, *Drosophila* has helped to solve the genetic control of immune system under infectious states (Buchon *et al*. 2014; Imler, 2014).

Drosophila immune system is divided, as is mammals', into two parts; cellular and humoral responses (Elrod-Erickson *et al*. 2000; Royet *et al*. 2003). At the cellular level, *Drosophila* is protected by cellular immunity through the actions of hemocytes, *Drosophila* blood cells, which exist in the form of plasmatocytes, lamellocytes, and crystal cells (Lemaitre & Hoffmann, 2007; Parsons & Foley, 2016). Plasmatocytes, spherical cells with a diameter of about 10 μm, function similarly to mammalian macrophages being responsible for the phagocytic elimination of invading bacteria (Chung & Kocks, 2011; Nonaka *et al*. 2013; Shiratsuchi *et al*. 2012) and viruses (Zhu & Zhang, 2013) as well as cells undergoing physiological (Franc *et al*. 1999; Nonaka *et al*. 2017; Nonaka *et al*. 2013) or virus-induced (Lamiable *et al*. 2016; Nainu *et al*. 2015) apoptosis. Extensive studies have clarified how *Drosophila* innate immune system involving plasmatocytes recognizes pathogens in a mechanism resembling humans' (Buchon *et al*. 2014; Gold & Brückner, 2015; Lemaitre & Hoffmann, 2007; Nainu *et al*. 2017; Wang *et al*. 2013). Also, the analysis of lamellocytes and crystal cells has provided information regarding a defense against parasitic infections and melanization processes (Lemaitre & Hoffmann, 2007). To induce humoral immune responses, *Drosophila* activates signaling pathways known as the Toll and Imd pathways that are connected with the induction of transcription, apoptosis, autophagy, and RNA interference (Buchon *et al*. 2014; De Gregorio *et al*. 2002; Karlikow *et al*. 2014; McPhee & Baehrecke, 2009; Merkling & van Rij, 2013; Myllymäki *et al*. 2014; Usmar *et al*. 2017; Valanne *et al*. 2011; Xu & Cherry, 2014; Zeidler *et al*. 2000). All these events lead to the production of soluble proteins that counteract pathogenic attacks, such as bacteria, fungi, and viruses.

Researchers have infected *Drosophila* with bacteria that cause human diseases (Panayidou *et al*. 2014) to examine the effectiveness of antibiotic compounds such as tetracycline and amoxicillin (Apidianakis & Rahme, 2009; Ben-Ami *et al*. 2013; Needham *et al*. 2004). Adopting this strategy, *Drosophila* is now used to test the activity of natural compounds derived from plants as antibacterial (Nainu *et al*. 2018) or antiviral (Ekowati *et al*. 2017) agents. A variety of *Drosophila* mutants enable researchers to test candidate compounds in immune-compromised animals in a rapid, simple, and economical manner.

4. *Drosophila* as a model of metabolic syndrome disorders

Obesity and related metabolic disorders such as

diabetes mellitus are still one of the highest causes of death in the world (Arroyo-Johnson & Mincey, 2016; Zheng *et al*. 2017). Seeing this trend, the discovery of more effective drugs is urgently needed. The generation of a *Drosophila* model of diabetes mellitus should be of tremendous help (Alfa & Kim, 2016; Graham & Pick, 2017). It should be noted that *Drosophila* does not have pancreatic organs, and its physiology is quite different from humans'. However, at the level of individual cells, there exists a huge functional resemblance to humans (Alfa & Kim, 2016). For example, *Drosophila* contains a protein named *Drosophila* insulin-like protein (DILP) equivalent to insulin (Alfa & Kim, 2016; Nässel *et al*. 2013). Destruction of DILP-producing cells causes an increase in the levels of glucose and lipids in hemolymph, body fluids of *Drosophila*. This means that *Drosophila* experiences diabetes-like symptoms, suggesting *Drosophila* as a suitable model animal for studying the pathophysiology of diabetes and related diseases (Alfa & Kim, 2016; Palanker Musselman *et al*. 2011; Pandey & Nichols, 2011; Rulifson *et al*. 2002). Reduced expression of DILP has negative effects on the growth of *Drosophila* making the size of larvae and adults smaller (Kannan & Fridell, 2013; Ruaud & Thummel, 2008; Rulifson *et al*. 2002). Therefore, body size is a potential phenotypic indicator in the screening of drug candidates against metabolic diseases (Pandey & Nichols, 2011). Furthermore, *Drosophila* possesses receptors homologous to sulfonylurea receptors in humans, which function to control a glucose balance. Thus, *Drosophila* can be used in high throughput screening of drug candidates anticipating a mechanism similar to glibenclamide or drugs in the sulfonylurea group (Pandey & Nichols, 2011).

Drosophila has been used in a study on the relationship between nutrition and obesity (Musselman & Kühnlein, 2018). To generate an obesity model, *Drosophila* is fed with a diet of high triglyceride content. Under such a diet condition, *Drosophila* dramatically gains weight and reduces movement, and eventually its life span is shortened (J. Hoffmann *et al*. 2013). Also, feeding *Drosophila* with a high content of fatty acids (derived from coconut oil) creates a phenotype resembling metabolic syndromes. Interestingly, a glucose status is influenced by the duration of feeding with saturated fatty acids: feeding for a short period decreases glucose levels associated with increased levels of DILP. In contrast, prolonged feeding induces an increase in the level of glucose and a decrease in insulin responses, as commonly found with patients suffering from type 2 diabetes mellitus (Birse *et al*. 2010). Certainly, the use of a *Drosophila* model in an effort to discover new drugs to treat metabolic syndromes is promising (Men *et al*. 2016; Pandey & Nichols, 2011; Smith *et al*. 2014).

PROSPECT OF *Drosophila* AS A MODEL ORGANISM IN DRUG DISCOVERY

At present, researchers have used *Drosophila* as an *in vivo* platform for screening drug candidates. An advantage of this insect is that we can obtain results in a short time at low cost (Pandey & Nichols, 2011). A research group led by Ross Cagan conducted a pre-clinical test of drug candidates with *Drosophila* without an *in vitro* test using cell cultures or other *in vitro* platforms (Vidal *et al*. 2005) and successfully identified Vandetanib (ZD6474) that was approved by US FDA for the treatment of medullary thyroid carcinoma in 2011.

When examining the pharmacological effects of new drug candidates, the route of administration needs to be chosen. There are several administration routes feasible with *Drosophila*, and a choice is dependent on at which developmental stages *Drosophila* is used. For example, embryos may be administered with drugs by a permeabilization method while larvae and adults are fed with food that contains drugs. Adult flies may also be administered with drugs in the form of vapor and by injection directly into the body cavity. In some cases, larve and adults are given drugs that are dissolved in glucose-containing water and absorbed in a filter paper (Pandey & Nichols, 2011).

The fruit fly *Drosophila melanogaster* is an ideal model animal in research toward drug discovery because it resembles humans in genetic materials, physiology, pathology, and reactions to medications. Various biological processes seen in human bodies can be studied using *Drosophila*, such as gene expression, reproduction, body development y, cell division and differentiation, cell death, energy metabolisms, and immunity. However, we had better be careful because none of model animals cannot be absolutely the same as humans. In fact, *Drosophila* and humans differ in the size and organization of body that sometimes become a limitation on the use of this model organism in research. For example, due to the absence of blood vessels in *Drosophila*, testing the effects of drugs

on hemostasis is rather difficult. Also, we are unable to discover drugs that function through study adaptive immunity that is absent in *Drosophila*.

CONCLUSION

Drosophila has been used as a model organism in genetic research for more than 100 years. Many researchers now consider *Drosophila* as a tool for generating human disease models and subsequent pharmacological testing of new drug candidates. Thanks to the availability of various genetic approaches and less time-consuming operations in generating mutants and transgenic genotypes, the use of *Drosophila* will be expanding and promising. We will identify and annotate many uncharacterized human genes using *Drosophila* as a surrogate creature. Also, we will rapidly complete the screening of candidate substances at a pre-clinical level and surely obtain novel drugs to cure intractable human diseases.

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REFERENCES

Adams, M.D., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatides, P.G., *et al*. (2000). The genome sequence of *Drosophila melanogaster*. *Science*. 287(5461): 2185-2195. doi: 10.1126/science.287.5461.2185

Alfa, R.W., & Kim, S.K. (2016). Using Drosophila to discover mechanisms underlying type 2 diabetes. *Dis Model Mech*. 9(4): 365-376. doi: 10.1242/dmm.023887

Apidianakis, Y., & Rahme, L.G. (2009). *Drosophila melanogaster* as a model host for studying *Pseudomonas aeruginosa* infection. *Nat Protoc*. 4(9): 1285-1294.

Arroyo-Johnson, C., & Mincey, K.D. (2016). Obesity epidemiology worldwide. *Gastroenterol Clin North Am*. 45(4): 571-579. doi: https://doi.org/10.1016/j.gtc.2016.07.012

Bellen, H.J., Tong, C., & Tsuda, H. (2010). 100 years of Drosophila research and its impact on vertebrate neuroscience: a history lesson for the future. *Nat Rev Neurosci*. 11: 514. doi: 10.1038/nrn2839

Ben-Ami, R., Watson, C.C., Lewis, R.E., Albert, N.D., Arias, C.A., Raad, I.I., *et al*. (2013). *Drosophila melanogaster* as a model to explore the effects of methicillin-resistant *Staphylococcus aureus* strain type on virulence and response to linezolid treatment. *Microb Pathog*. 55: 16-20. doi: https://doi.org/10.1016/j.micpath.2012.11.012

Bier, E. (2005). Drosophila, the golden bug, emerges as a tool for human genetics. *Nat Rev Genet*. 6: 9. doi: 10.1038/nrg1503

Birse, R.T., Choi, J., Reardon, K., Rodriguez, J., Graham, S., Diop, S., *et al*. (2010). High fat diet-induced obesity and heart dysfunction is regulated by the TOR pathway in Drosophila. *Cell Metab*. 12(5): 533-544. doi: 10.1016/j.cmet.2010.09.014

Bolon, B. (2004). Genetically engineered animals in drug discovery and development: A maturing resource for toxicologic research. *Basic Clin Pharmacol Toxicol*. 95(4): 154-161. doi: doi:10.1111/j.1742-7843.2004.pto950402.x

Bonner, J.M., & Boulianne, G.L. (2011). Drosophila as a model to study age-related neurodegenerative disorders: Alzheimer's disease. *Exp Gerontol*. 46(5): 335-339. doi: https://doi.org/10.1016/j.exger.2010.08.004

Brad, B., & Elizabeth, G. (2002). Use of genetically engineered mice in drug discovery and development: Wielding Occam's razor to prune the product portfolio. *Int J Toxicol*. 21(1): 55-64. doi: 10.1080/10915810252826019

Brandt, A., & Vilcinskas, A. (2013). The fruit fly *Drosophila melanogaster* as a model for aging research. In A. Vilcinskas (Ed.), *Yellow Biotechnology I: Insect Biotechnologie in Drug Discovery and Preclinical Research* (pp. 63-77). Berlin, Heidelberg: Springer Berlin Heidelberg.

Breyer, M.D., Look, A.T., & Cifra, A. (2015). From bench to patient: model systems in drug discovery. *Dis Model Mech*. 8(10): 1171-1174. doi: 10.1242/dmm.023036

Brumby, A.M., & Richardson, H.E. (2005). Using

Drosophila melanogaster to map human cancer pathways. *Nat Rev Cancer*. 5: 626-639. doi: 10.1038/nrc1671

Buchon, N., Silverman, N., & Cherry, S. (2014). Immunity in *Drosophila melanogaster* - from microbial recognition to whole-organism physiology. *Nat Rev Immunol*. 14: 796-810. doi: 10.1038/nri3763

Carroll, S.B. (1995). Homeotic genes and the evolution of arthropods and chordates. *Nature*, 376: 479-485. doi: 10.1038/376479a0

Chien, S., Reiter, L.T., Bier, E., & Gribskov, M. (2002). Homophila: human disease gene cognates in Drosophila. *Nucleic Acids Res*. 30(1): 149-151.

Christofi, T., & Apidianakis, Y. (2013). Drosophila and the hallmarks of cancer. In A. Vilcinskas (Ed.), *Yellow Biotechnology I: Insect Biotechnologie in Drug Discovery and Preclinical Research* (pp. 79-110). Berlin, Heidelberg: Springer.

Christofori, G., & Semb, H. (1999). The role of the cell-adhesion molecule E-cadherin as a tumour-suppressor gene. *Trend Biochem Sci*. 24(2): 73-76. doi: 10.1016/S0968-0004(98)01343-7

Chung, Y.-S. A., & Kocks, C. (2011). Recognition of pathogenic microbes by the Drosophila phagocytic pattern recognition receptor Eater. *J Biol Chem*. 286(30): 26524-26532. doi: 10.1074/jbc.M110.214007

De Gregorio, E., Spellman, P.T., Tzou, P., Rubin, G.M., & Lemaitre, B. (2002). The Toll and Imd pathways are the major regulators of the immune response in Drosophila. *EMBO J*. 21(11): 2568-2579. doi: 10.1093/emboj/21.11.2568

Dubnau, J., & Tully, T. (1998). Gene discovery in Drosophila: New insights for learning and memory. *Annu Rev Neurosci*. 21(1): 407-444. doi: 10.1146/annurev.neuro.21.1.407

Ekowati, H., Arai, J., Damana Putri, A.S., Nainu, F., Shiratsuchi, A., & Nakanishi, Y. (2017). Protective effects of *Phaseolus vulgaris* lectin against viral infection in Drosophila. *Drug Discov Ther*. 11(6): 329-335. doi: 10.5582/ddt.2017.01071

Elrod-Erickson, M., Mishra, S., & Schneider, D. (2000). Interactions between the cellular and humoral immune responses in Drosophila. *Curr Biol*. 10(13): 781-784. doi: 10.1016/S0960-9822(00)00569-8

Fernández‐Hernández, I., Scheenaard, E., Pollarolo, G., & Gonzalez, C. (2016). The translational relevance of *Drosophila* in drug discovery. *EMBO Rep*. 17(4): 471-472. doi: 10.15252/embr.201642080

Franc, N.C., Heitzler, P., Ezekowitz, R.A & White, K. (1999). Requirement for Croquemort in phagocytosis of apoptotic cells in Drosophila. *Science*. 284(5422): 1991-1994. doi: 10.1126/science.284.5422.1991

Giacomotto, J., & Ségalat, L. (2010). High‐throughput screening and small animal models, where are we? *Br J Pharmacol*. 160(2): 204-216. doi: doi:10.1111/j.1476-5381.2010.00725.x

Gibson, G., & Muse, S. (2009). *A primer of genome science* (3rd ed.). New York: Oxford University Press Inc.

Gold, K. S., & Brückner, K. (2015). Macrophages and cellular immunity in *Drosophila melanogaster*. *Sem Immunol*. 27(6): 357-368. doi: 10.1016/j.smim.2016.03.010

Gonzalez, C. (2013). *Drosophila melanogaster*: a model and a tool to investigate malignancy and identify new therapeutics. *Nat Rev Cancer*. 13: 172-183. doi: 10.1038/nrc3461

Graham, P., & Pick, L. (2017). Drosophila as a model for diabetes and diseases of insulin resistance. *Curr Top Dev Biol*. 121: 397-419. doi: 10.1016/bs.ctdb.2016.07.011

Hales, K.G., Korey, C.A., Larracuente, A.M., & Roberts, D.M. (2015). Genetics on the fly: A primer on the Drosophila model system. *Genetics*. 201(3): 815-842. doi: 10.1534/genetics.115.183392

He, Y., & Jasper, H. (2014). Studying aging in *Drosophila*. *Methods*. 68(1): 129-133. doi: 10.1016/j.ymeth.2014.04.008

Hoffmann, J., Romey, R., Fink, C., & Roeder, T. (2013). *Drosophila* as a model to study metabolic disorders. In A. Vilcinskas (Ed.), *Yellow Biotechnology I: Insect Biotechnologie in Drug Discovery and Preclinical Research* (pp. 41-61). Berlin, Heidelberg: Springer

Hoffmann, J.A. (2003). The immune response of *Drosophila*. *Nature*, 426: 33-38. doi: 10.1038/nature02021

Imler, J.-L. (2014). Overview of *Drosophila* immunity: A historical perspective. *Dev Comp Immunol*. 42(1): 3-15. doi: https://doi.org/10.1016/j.dci.2013.08.018

Kannan, K., & Fridell, Y.W. (2013). Functional implications of *Drosophila* insulin-like peptides in metabolism, aging, and dietary restriction. *Front Physiol*. 4(288). doi: 10.3389/fphys.2013.00288

Karlikow, M., Goic, B., & Saleh, M.C. (2014). RNAi and antiviral defense in *Drosophila*: Setting up a systemic immune response. *Dev Comp Immunol*. 42(1): 85-92. doi: https://doi.org/10.1016/j.dci.2013.05.004

Konopka, R.J., & Benzer, S. (1971). Clock mutants of *Drosophila melanogaster*. *Proc Natl Acad Sci*. 68(9): 2112-2116. doi: 10.1073/pnas.68.9.2112

Kumar, J.P. (2012). Building an ommatidium one cell at a time. *Dev Dyn*. 241(1): 136-149. doi: 10.1002/dvdy.23707

Lamiable, O., Arnold, J., de Faria, I.J.D.S., Olmo, R.P., Bergami, F., Meignin, C., Hoffman, J.A., Marques, J.T., & Imler, J.L. (2016). Analysis of the contribution of hemocytes and autophagy to *Drosophila* antiviral immunity. *J Virol*. 90(11): 5415-5426. doi: 10.1128/JVI.00238-16

Lemaitre, B., & Hoffmann, J. (2007). *The host defense of Drosophila melanogaster*. *Annu Rev Immunol*. 25(1): 697-743. doi: 10.1146/annurev.immunol.25.022106.141615

Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J.M., & Hoffmann, J.A. (1996). The dorsoventral regulatory gene cassette *spätzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults. *Cell*. 86(6): 973-983. doi: https://doi.org/10.1016/S0092-8674(00)80172-5

Li, H., & Garza, D. (2004). *Drosophila* as a tool for drug discovery. In P. M. Carroll & K. Fitzgerald (Eds.), *Model Organisms in Drug Discovery* (pp. 81-117): Wiley.

Mackay, T.F.C. (2010). Mutations and quantitative genetic variation: lessons from *Drosophila*. *Philos Trans R Soc Lond B Biol Sci*. 365(1544): 1229-1239. doi: 10.1098/rstb.2009.0315

Markow, T.A. (2015). The secret lives of Drosophila flies. *eLife*. 4: e06793. doi: 10.7554/eLife.06793

Marygold, S.J., Crosby, M.A., & Goodman, J.L. (2016). Using FlyBase, a database of *Drosophila* genes and genomes. In C. Dahmann (Ed.), *Drosophila: Methods and Protocols* (pp. 1-31). New York, NY: Springer New York.

Matthews, K.A., Kaufman, T.C., & Gelbart, W.M. (2005). Research resources for *Drosophila*: the expanding universe. *Nat Rev Genet*. 6: 179-193. doi: 10.1038/nrg1554

McGonigle, P., & Ruggeri, B. (2014). Animal models of human disease: Challenges in enabling translation. *Biochem Pharmacol*. 87(1): 162-171. doi:https://doi.org/10.1016/j.bcp.2013.08. 006

McPhee, C.K., & Baehrecke, E.H. (2009). Autophagy in *Drosophila melanogaster*. *Biochim Biophys Acta*. 1793(9): 1452-1460. doi: https://doi.org/10.1016/j.bbamcr.2009.02.009

Medzhitov, R., Preston-Hurlburt, P., & Janeway Jr, C.A. (1997). A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature*. 388: 394-397. doi: 10.1038/41131

Meier, P., Finch, A., & Evan, G. (2000). Apoptosis in development. *Nature*. 407: 796-801. doi: 10.1038/35037734

Men, T.T., Thanh, D.N.V., Yamaguchi, M., Suzuki, T., Hattori, G., Arii, M., *et al*. (2016). A *Drosophila* model for screening antiobesity agents. *BioMed Res Int*. 2016: 10. doi: 10.1155/2016/6293163

Merkling, S.H., & van Rij, R.P. (2013). Beyond RNAi: Antiviral defense strategies in Drosophila and mosquito. *J Insect Physiol*. 59(2): 159-170. doi: https://doi.org/10.1016/j.jinsphys.2012.07. 004

Miles, W.O., Dyson, N.J., & Walker, J.A. (2011). Modeling tumor invasion and metastasis in *Drosophila*. *Dis Model Mech*. 4(6): 753-761. doi: 10.1242/dmm.006908

Musselman, L.P., & Kühnlein, R.P. (2018). *Drosophila* as a model to study obesity and metabolic disease. *J Exp Biol*. 221(Suppl 1). doi: 10.1242/jeb.163881

Myllymäki, H., Valanne, S., & Rämet, M. (2014). The *Drosophila* Imd signaling pathway. *J Immunol*. 192(8): 3455-3462. doi: 10.4049/jimmunol.1303309

Nainu, F., Asri, R.M., Arsyad A., Manggau, M.A., & Amir, M.N. (2018). *In vivo* antibacterial activity of green algae *Ulva reticulata* against *Staphylo-*

coccus aureus in *Drosophila* model of infection. *Pharmacog J*. 10(5): 993-997. doi:10.5530/pj.2018.5.169

Nainu, F., Shiratsuchi, A., & Nakanishi, Y. (2017). Induction of apoptosis and subsequent phagocytosis of virus-infected cells as an antiviral mechanism. *Front Immunol*. 8(1220). doi: 10.3389/fimmu.2017.01220

Nainu, F., Tanaka, Y., Shiratsuchi, A., & Nakanishi, Y. (2015). Protection of insects against viral infection by apoptosis-dependent phagocytosis. *J Immunol*. 195(12): 5696-5706. doi: 10.4049/jimmunol.1500613

Nakanishi, Y., Nagaosa, K., & Shiratsuchi, A. (2011). Phagocytic removal of cells that have become unwanted: Implications for animal development and tissue homeostasis. *Dev Growth Differ*. 53(2): 149-160. doi: doi:10.1111/j.1440-169X.2010.01224.x

Nässel, D.R., Kubrak, O.I., Liu, Y., Luo, J., & Lushchak, O.V. (2013). Factors that regulate insulin producing cells and their output in *Drosophila*. *Front Physiol*. 4: 252. doi: 10.3389/fphys.2013.00252

Needham, A.J., Kibart, M., Crossley, H., Ingham, P.W., & Foster, S.J. (2004). *Drosophila melanogaster* as a model host for *Staphylococcus aureus* infection. *Microbiol*. 150(7): 2347-2355. doi: doi:10.1099/mic.0.27116-0

Nonaka, S., Ando, Y., Kanetani, T., Hoshi, C., Nakai, Y., Nainu, F., *et al*. (2017). Signaling pathway for phagocyte priming upon encounter with apoptotic cells. *J Biol Chem*. 292(19): 8059-8072. doi: 10.1074/jbc.M116.769745

Nonaka, S., Nagaosa, K., Mori, T., Shiratsuchi, A., & Nakanishi, Y. (2013). Integrin αPS3/βv-mediated phagocytosis of apoptotic cells and bacteria in Drosophila. *J Biol Chem*. 288(15): 10374-10380. doi: 10.1074/jbc.M113.451427

Olivier, J.P., Raabe, T., Henkemeyer, M., Dickson, B., Mbamalu, G., Margolis, B., *et al*. (1993). A *Drosophila* SH2-SH3 adaptor protein implicated in coupling the sevenless tyrosine kinase to an activator of Ras guanine nucleotide exchange, Sos. *Cell*. 73(1): 179-191. doi: 10.1016/0092-8674(93)90170-U

Palanker Musselman, L., Fink, J.L., Narzinski, K., Ramachandran, P.V., Sukumar Hathiramani, S., Cagan, R.L., *et al*. (2011). A high-sugar diet produces obesity and insulin resistance in wild-type *Drosophila*. *Dis Model Mech*. 4(6): 842-849. doi: 10.1242/dmm.007948

Panayidou, S., Ioannidou, E., & Apidianakis, Y. (2014). Human pathogenic bacteria, fungi, and viruses in *Drosophila*: Disease modeling, lessons, and shortcomings. *Virulence*. 5(2): 253-269. doi: 10.4161/viru.27524

Panchal, K., & Tiwari, A.K. (2017). *Drosophila melanogaster* "a potential model organism" for identification of pharmacological properties of plants/plant-derived components. *Biomed Pharmacother*. 89: 1331-1345. doi: https://doi.org/10.1016/j.biopha.2017.03.001

Pandey, U.B., & Nichols, C.D. (2011). Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. *Pharmacol Rev*. 63(2): 411-436. doi: 10.1124/pr.110.003293

Parsons, B., & Foley, E. (2016). Cellular immune defenses of *Drosophila melanogaster*. *Dev Comp Immunol*. 58: 95-101. doi: https://doi.org/10.1016/j.dci.2015.12.019

Patel, S., & Prokop, A. (2017). The Manchester Fly Facility: Implementing an objective-driven long-term science communication initiative. *Semin Cell Dev Biol*. 70: 38-48. doi: https://doi.org/10.1016/j.semcdb.2017.06.004

Pearson, J.C., Lemons, D., & McGinnis, W. (2005). Modulating Hox gene functions during animal body patterning. *Nat Rev Genet*. 6: 893-904. doi: 10.1038/nrg1726

Rajan, A., & Perrimon, N. (2013). Of flies and men: insights on organismal metabolism from fruit flies. *BMC Biol*. 11: 38-38. doi: 10.1186/1741-7007-11-38

Reaume, C.J., & Sokolowski, M.B. (2006). The nature of *Drosophila melanogaster*. *Curr Biol*. 16(16): R623-R628. doi: https://doi.org/10.1016/j.cub.2006.07.042

Reiter, L.T. (2005). *Drosophila* as a model for human diseases *eLS* (Vol. 1): Wiley-Blackwell.

Reiter, L.T., Potocki, L., Chien, S., Gribskov, M.,

& Bier, E. (2001). A systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster*. *Genome Res*. 11(6): 1114-1125. doi: 10.1101/gr.169101

Rieder, L.E., & Larschan, E.N. (2014). Wisdom from the Fly. *Trend Genet*. 30(11): 479-481. doi: 10.1016/j.tig.2014.08.003

Royet, J., Meister, M., & Ferrandon, D. (2003). Humoral and cellular responses in Drosophila innate immunity. In R. A. B. Ezekowitz & J. A. Hoffmann (Eds.), *Innate Immunity* (pp. 137-153). Totowa, NJ: Humana Press.

Ruaud, A.F., & Thummel, C.S. (2008). Serotonin and insulin signaling team up to control growth in *Drosophila*. *Genes Dev*. 22(14): 1851-1855. doi: 10.1101/gad.1700708

Rudrapatna, V.A., Cagan, R.L., & Das, T.K. (2012). *Drosophila* cancer models. *Dev Dyn*. 241(1): 107-118. doi: 10.1002/dvdy.22771

Ruggeri, B.A., Camp, F., & Miknyoczki, S. (2014). Animal models of disease: Pre-clinical animal models of cancer and their applications and utility in drug discovery. *Biochem Pharmacol*. 87(1): 150-161. doi: https://doi.org/10.1016/j.bcp.2013.06.020

Rulifson, E.J., Kim, S.K., & Nusse, R. (2002). Ablation of insulin-producing neurons in flies: Growth and diabetic phenotypes. *Science*. 296(5570): 1118-1120. doi: 10.1126/science.1070058

Shaw, R.J., & Cantley, L.C. (2006). Ras, PI(3)K and mTOR signalling controls tumour cell growth. *Nature*. 441: 424-430. doi: 10.1038/nature04869

Shiratsuchi, A., Mori, T., Sakurai, K., Nagaosa, K., Sekimizu, K., Lee, B.L., *et al*. (2012). Independent recognition of *Staphylococcus aureus* by two receptors for phagocytosis in Drosophila. *J Biol Chem*. 287(26): 21663-21672. doi: 10.1074/jbc.M111.333807

Simon, M.A., Bowtell, D.D.L., Dodson, G.S., Laverty, T.R., & Rubin, G.M. (1991). Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the sevenless protein tyrosine kinase. *Cell*. 67(4): 701-716. doi: 10.1016/0092-8674(91)90065-7

Smith, W.W., Thomas, J., Liu, J., Li, T., & Moran, T.H. (2014). From fat fruitfly to human obesity. *Physiol Behav*. 0: 15-21. doi: 10.1016/j.physbeh.2014.01.017

Snaith, M.R., & Törnell, J. (2002). The use of transgenic systems in pharmaceutical research. *Brief Funct Genomics*. 1(2): 119-130. doi: 10.1093/bfgp/1.2.119

Sonoshita, M., & Cagan, R.L. (2017). Modeling human cancers in *Drosophila*. In L. Pick (Ed.), *Current Topics in Developmental Biology* (Vol. 121, pp. 287-309): Academic Press.

Strange, K. (2016). Drug discovery in fish, flies, and worms. *ILAR J*. 57(2): 133-143. doi: 10.1093/ilar/ilw034

Sun, Y., Yolitz, J., Wang, C., Spangler, E., Zhan, M., & Zou, S. (2013). Aging studies in *Drosophila Melanogaster*. In T. O. Tollefsbol (Ed.), *Biological Aging: Methods and Protocols* (pp. 77-93). Totowa, NJ: Humana Press.

Ugur, B., Chen, K., & Bellen, H.J. (2016). Drosophila tools and assays for the study of human diseases. *Dis Model Mech*. 9(3): 235-244. doi: 10.1242/dmm.023762

Usmar, U., Arfiansyah, R., & Nainu, F. (2017). Sensor asam nukleat sebagai aktivator imunitas intrinsik terhadap patogen intraseluler. *Galenika Journal of Pharmacy*. 3(2): 174-190.

Valanne, S., Wang, J.H., & Rämet, M. (2011). The Drosophila Toll signaling pathway. *J Immunol*. 186(2): 649-656. doi: 10.4049/jimmunol.1002302

Venken, K.J.T., & Bellen, H.J. (2007). Transgenesis upgrades for *Drosophila melanogaster*. *Development*. 134(20): 3571-3584. doi: 10.1242/dev.005686

Vidal, M., Wells, S., Ryan, A., & Cagan, R. (2005). ZD6474 suppresses oncogenic RET isoforms in a Drosophila model for type 2 multiple endocrine neoplasia syndromes and papillary thyroid carcinoma. *Cancer Res*. 65(9): 3538-3541. doi: 10.1158/0008-5472.can-04-4561

Vogel, H.G., & Vogel, W.H. (2013). *Drug discovery and evaluation*: *Pharmacological assays*: Springer Berlin Heidelberg.

Wang, L., Kounatidis, I., & Ligoxygakis, P. (2013). Drosophila as a model to study the role of blood cells in inflammation, innate immunity and cancer. *Front Cell Infect Microbiol*. 3: 113. doi:

10.3389/fcimb.2013.00113

Wangler, M.F., & Bellen, H.J. (2017). *In vivo* animal modeling: Drosophila In F. Y. L. Saldanha & M. Jalali (Eds.), *Basic Science Methods for Clinical Researchers* (pp. 211-234). Boston: Academic Press.

Wangler, M.F., Yamamoto, S., & Bellen, H.J. (2015). Fruit flies in biomedical research. *Genetics*. 199(3): 639-653. doi: 10.1534/genetics.114.171785

Willoughby, L.F., Schlosser, T., Manning, S.A., Parisot, J.P., Street, I.P., Richardson, H.E., *et al*. (2013). An *in vivo* large-scale chemical screening platform using Drosophila for anti-cancer drug discovery. *Dis Model Mech*. 6(2): 521-529. doi: 10.1242/dmm.009985

Xu, J., & Cherry, S. (2014). Viruses and antiviral immunity in Drosophila. *Dev Comp Immunol*. 42(1): 10.1016/j.dci.2013.1005.1002. doi: 10.1016/j.dci.2013.05.002

Yamamoto, S., Jaiswal, M., Charng, W.L., Gambin, T., Karaca, E., Mirzaa, G., *et al*. (2014). A Drosophila genetic resource of mutants to study mechanisms underlying human genetic diseases. *Cell*. 159(1): 200-214. doi: 10.1016/j.cell.2014.09.002

Zeidler, M.P., Bach, E.A., & Perrimon, N. (2000). The roles of the Drosophila JAK/STAT pathway. *Oncogene*. 19: 2598. doi: 10.1038/sj.onc.1203482

Zheng, Y., Ley, S.H., & Hu, F.B. (2017). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol*. 14: 88-98. doi: 10.1038/nrendo.2017.151

Zhu, F., & Zhang, X. (2013). The Wnt signaling pathway is involved in the regulation of phagocytosis of virus in Drosophila. *Sci Rep*. 3: 2069. doi: 10.1038/srep02069

Zuberi, A., & Lutz, C. (2016). Mouse models for drug discovery: Can new tools and technology improve translational power? *ILAR J*. 57(2): 178-185. doi: 10.1093/ilar/ilw021.