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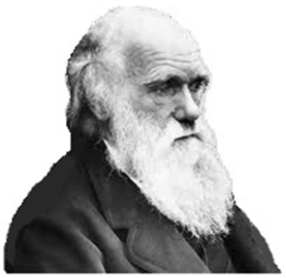

World News of Natural Sciences

An International Scientific Journal

WNOFNS 36 (2021) 42-59 EISSN 2543-5426

Drosophila melanogaster (Meigen, 1830): A Potential Model for Human Diseases

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ABSTRACT

Over some time, *Drosophila melanogaster* (Meigen, 1830), commonly called fruit fly, has been used as a model organism in both scientific and medical research. *Drosophila* in comparison with other mammalian species shares some basic features like physiological, biological, biochemical, and neurological resemblances which make them suitable for use for biomedical research. Fruit fly can be maintained efficiently at a reduced cost in the laboratory, and it is endorsed as an alternative model compared to other vertebrates. It is confirmed and documented that almost 75 % of human disease-causing genes have functional similarities in *Drosophila*. Nevertheless, the use of *D. melanogaster* as a model organism was not narrowed to genetic research only, but several experiments. The use of this organism as a model for human diseases has also led to findings like neurodegenerative diseases, Huntington's disease, spinocerebellar ataxia type 3, cancer, cardiovascular, inflammation and infectious diseases, and metabolic disorders. The fly is used as an ideal model organism for neurodegenerative disease studies such as Alzheimer's and Parkinson's, which have become more predominant in today's aging population due to its complex nervous system which conserved neurological function, and the human disease-related loci. In this review, we presented and discussed *Drosophila melanogaster* as a model to study several human diseases.

***Keywords*:** *Drosophila melanogaster*, human diseases, neurodegenerative disease, Huntington's disease, spinocerebellar ataxia type 3

(Received 12 February 2021; Accepted 01 March 2021; Date of Publication 02 March 2021)

1. INTRODUCTION

Drosophila melanogaster, an arthropod, is a dipteran (a member of an order of insects containing true flies or two-winged) insect, belonging to the family Drosophilidae. This fly was presented about 100 years ago as a model in biology and it was conclusive for the development of genetic and other related fields (Sepel and Loreto, 2010). Various experiments with this fly have also led to findings in neurodegenerative diseases such as Alzheimer's and Parkinson's (Hirth, 2010), Huntington's disease (Zhang *et al.*, 2009), Spinocerebellar ataxia type 3 (Paulson *et al.*, 2000), cancer (Nagaraja and Banerjee, 2004), cardiovascular (Ocorr *et al*., 2007), inflammation and infectious diseases (Hirth, 2010), and metabolic disorders (Pendse *et al.*, 2013).

Owing to the successful results in the use of *Drosophila* in experimental studies, it has met the standard of the European Centre for the Validation of Alternative Methods (ECVAM): Reduction, Refinement and Replacement (3Rs) of laboratory animal usage (Festing *et al*., 1999). *D. melanogaster* as a model raises few ethical concerns and its genome can be easily manipulated in the study of a particular gene of interest under a defined condition. Interestingly, the post-genomic sequencing of *D. melanogaster* generated a great deal of attention to biomedical researchers because it revealed functional conservation of the majority of the genes present in mammals. As a result of this, it has been used to obtain mechanistic insights into human diseases. Thus, its use in toxicological studies will continue to generate valuable data.

D. melanogaster shows anatomical characteristic features such as compound eyes and wings. It has a lifecycle of between 40-120 days depending on environmental stress conditions (e.g., population density and temperature) and diet. A diet such as cornmeal extends the life expectancy of the fly, while diets with high quantities of cholesterol and saccharides (free available carbohydrates) can reduce lifespan (Hirth, 2010). Additionally, overpopulation has been shown to reduce the lifespan of the insect (Joshi and Mueller, 1997). The resemblances of molecular processes involved in the control of lifespan and aging between *Drosophila melanogaster* and human, as well as a good degree of genetic homology between this species, makes the fruit fly a fascinating model system for humans' diseases.

Of particular significance, over 65-70% of human disease genes can be found in *Drosophila* (Reiter *et al*., 2001; Pandey and Nichols, 2011; Poddighe *et al*., 2013), which makes it an important model to understand not only how the genes induce diseases, but also the finding of the relation of such genes to diseases (Fortini *et al*., 2000; Fortini and Bonini, 2000). When compared with other models, the fly undergoes rapid generation time, easy to use, and easy to conserve in the laboratory in large quantity due to its short lifecycle and tiny body size.

Fruit fly has a rapid life cycle of single fertile mating pair which can produce hundreds of genetically alike offsprings within 10 to 12 days at 25°C (Figure 1). Another fascinating feature of *Drosophila* is that it can be used as a multiple-model organism. Hence, its adult, pupa, larvae, and embryo can be used as models in different human disease settings. As such, the embryo and the pupa can be used as models in developmental human disease studies, whereas the larva can be used to study behavioral and physiological processes. Fascinatingly, the adult of *Drosophila melanogaster* possesses complex and sophisticated systems. It has some structures that can mimic the corresponding functions of the mammalian lung, heart, gut, kidney, and reproductive tract.

Additionally, the fruit fly's brain possesses more than 100,000 neurons that are vital in circadian rhythms, feeding, memory, courtship, aggression, and flight navigation. Essentially,

Drosophila melanogaster responds to numerous central nervous system drugs in a similar way to mammals (Nichols *et al*., 2002; Rothenfluh and Heberlein, 2002; Satta *et al*., 2003; Wolf and Heberlein, 2003; Andretic *et al*., 2008). This review aimed at concisely showing *Drosophila melanogaster* as a potential model for human diseases.

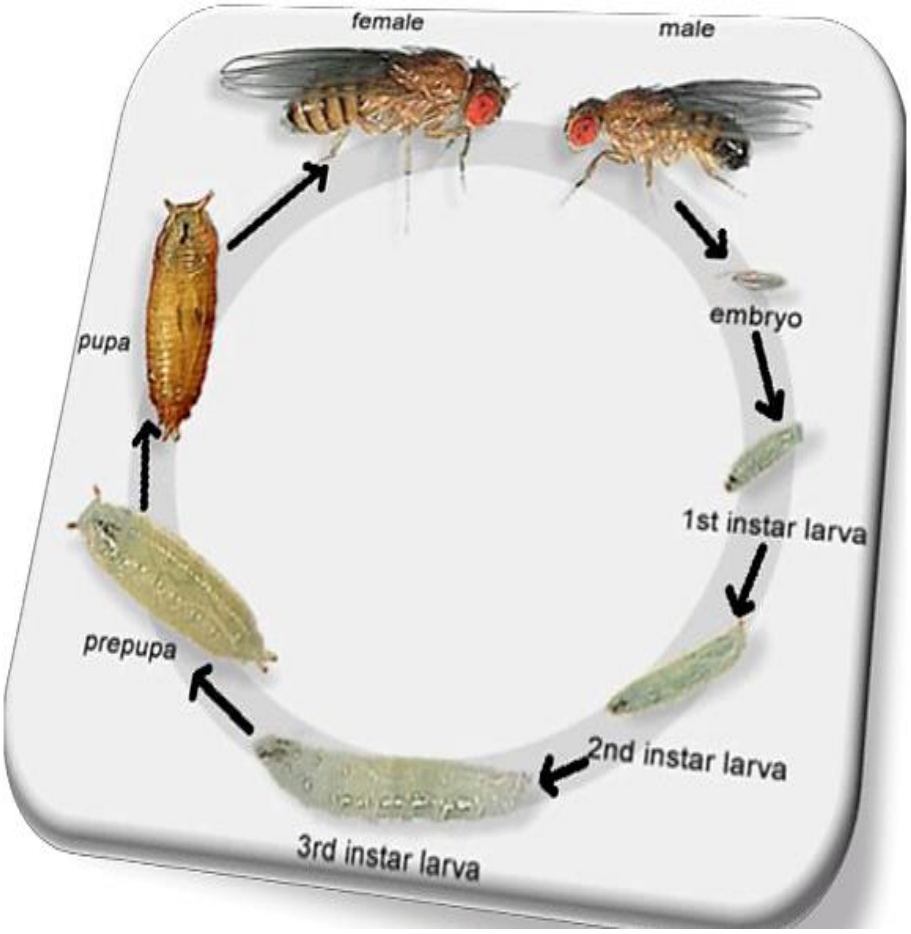

Figure 1. Life Cycle of *Drosophila melanogaster*. (Abolaji *et al*., 2013)

2. THE HISTORY OF FRUIT FLY

The history of fruit fl*ies* is over a century in science. Consequently, it is tough to do a comprehensive report in this review. Nevertheless, it is significant to highlight some important historical aspects of *D. melanogaster*. Charles William Woodworth is the first person to widely use the fly, who also recommended its use to William E. Castle. Its usage has subsequently spread intensely. *D. melanogaster* has been used to find out numerous landmark findings in genetics. The concept that the chromosomes carried hereditary traits was first developed in this fly. In fact, in 1994, Christiane Nusslein, Ed Lewis, and Eric Weischaus were awarded a Nobel Prize for Physiology and Medicine for their cutting-edge studies on fruit fl*ies* that led to findings ranging from outlining gene structure to the identification of vital genes involved in

embryogenesis. Afterward, these genes have been identified to be related to normal mammalian development. *D. melanogaster* is the first major complex organism to have its genome sequenced completely (Adams *et al*., 2000). This incidence vastly brings to face the detected homologies between the fly and the human genome when it was sequenced a few years later. This later bolstered the use of the fly as a model in human diseases and to understand human biology and disease processes. The fly has continued to gain the attention of biomedical researchers because molecular studies are often first revealed in simple organism models, such as fly, and later interpreted to mammalian systems (Bhan and Nichols, 2011).

3. THE CULTURE AND MEDIA OF *DROSOPHILA*

To integrate *D. melanogaster* for human diseases studies, it is important to conserve cultures of flies for backup as their lifespan is short. For the ease of culturing and transferring the flies, bottles and vials are desirable. It is also important to wholly wash and sterilize the bottles and vials to prevent contamination of diseases. Drosophila media have many standard formulations. In our laboratory for example, we preserve and rear flies on cornmeal medium (1%, weight/volume brewer's yeast; 1%, weight/volume agar; 2%, weight/volume sucrose; 1%, weight/volume powdered milk;; 0.08%, weight/volume nipagin/methylparaben) at constant humidity and temperature (60% relative humidity; 23+1^{o}C respectively) under 12 h light/dark cycle. Peng *et al*. (2009) and Li *et al*. (2007) have used basal diets containing 105 g of cornmeal, 105 g of glucose, 21 g of yeast, and 13 g of agar, then, 0.4% of Ethyl 4-hydroxybenzoate was added to the diet to prevent mold growth.

4. RESULT

4. 1. The potential uses of the fruit fly as human diseases model

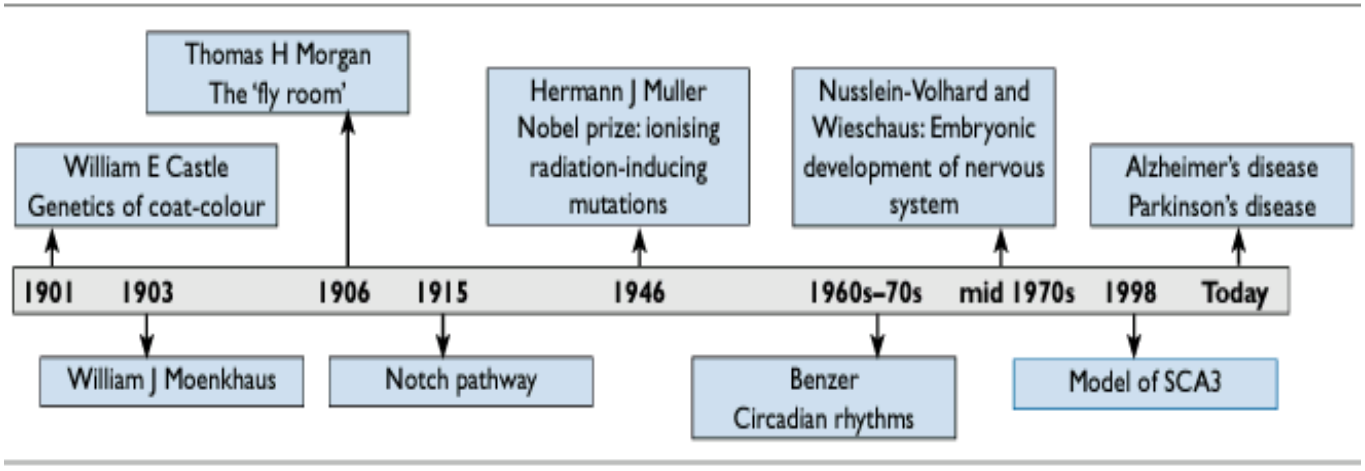

Figure 2. Timeline of the use of *Drosophila melanogaster* in the history of scientific research. (Stephenson and Metcalfe, 2013)

4. 2. Cancer

At one time, cancer research was conducted absolutely in mammalian cell-based systems ranging from tissue culture to whole-animal studies. In recent times, *D. melanogaster* has been gradually used as a model system. Possibly, one of the greatest contributions of the fly to the study of cancer biology was the exposition of the Ras signal transduction cascade more than 20 years ago in the fly visual system (Simon *et al.*, 1991; Olivier *et al.*, 1993; Nagaraj and Banerjee, 2004). Mammalian cells are found to preserve each of the major components of this pathway. At a basic level, cancer can be explained as the misregulation of signaling events in a cell that leads to abnormal proliferation and growth.

Based on the nature and type of cancer, the underlying mechanisms of the abnormal proliferation are wide-ranged and often remain vague. Hence, potential therapeutics are likely to be dependent upon a thorough understanding of individual types of cancers. The mainstream of cancer in human are derived from epithelial cells (Christofori and Semb, 1999), making these types of tumors significant targets for treatments. Accordingly, several models of *Drosophila* are being developed to study epithelial cell-derived cancers that could and are being translated to a discovery platform. These models include not only proliferative phenotypes but invasive and metastatic ones as well. The challenge here is to develop effective screening models that can identify agents and can prevent or inhibit proliferation and metastasis. One active strategy has been to misexpress either the *Drosophila melanogaster* version of a human signaling molecule linked to tumors or the human protein itself, in the eye of the fly.

The repeating “crystalline” nature of the eye of fruit fly makes it highly vulnerable to slight perturbations in development, which usually manifests as a rough or disorganized phenotype easily scored by simple observation (Cagan and Ready, 1989). For instance, Cagan and colleagues (Vidal and Cagan, 2006) misexpressed the fly homolog for the Ret receptor tyrosine kinase (implicated in human multiple endocrine neoplasia type 2), dRet, in a constitutively active form and produced a coarse eye phenotype. This fly was used to perform both modifier screens to identify interacting factors as well as to check the efficacy of a small molecule inhibitor of Ret in vivo (Vidal and Cagan, 2006). Additional epithelial models via morphological changes in adult structural phenotypes have also been developed for the discovery of molecules targeting the E-cadherin (Pereira et al., 2006), and EGF receptor/ras pathway (Aritakula and Ramasamy, 2008). Alternative methods have involved higher throughput strategies using larva and pupae.

For instance, one exciting model is a high-throughput platform examining pupal viability as a degree of tumor suppression. In this model, invasive tumors and ultimately cell death at the pupal phase are produced from the expression of a constitutively active form of Ras and a mutant of the tumor suppressor writing together in imaginal discs (Wu et al., 2010; Humbert *et al.*, 2008; Pagliarini and Xu, 2003). *Drosophila* was also engineered so that the tumors express the marker protein Granulin-Epithelin Precursor (GFP), allowing for visual quantification of tumor size and metastasis. Assays are conducted in 96-well microtiter plates with a small number of larva seeded per well, with drug present in the media. After 5 days, sucrose solution is added to the wells, and the dead larvae float to the top, where the GFP intensity, as a measure of tumor growth, can be estimated by microscopy.

Another high throughput screening system relies upon flies with a gain of function Raf or a dominant-negative allele of Notch, that each exhibit abnormal cell growth of midgut epithelium as a model for asymmetric stem cell division-related cancers (Januschke and Gonzalez, 2008; Micchelli and Perrimon, 2006). For this assay, Raf or Notch mutant flies that

express luciferase in gut epithelial cells are maintained on 96-well microtiter plates on media containing test drug, and then homogenized and assayed for luciferase activity as a measure of abnormal proliferation.

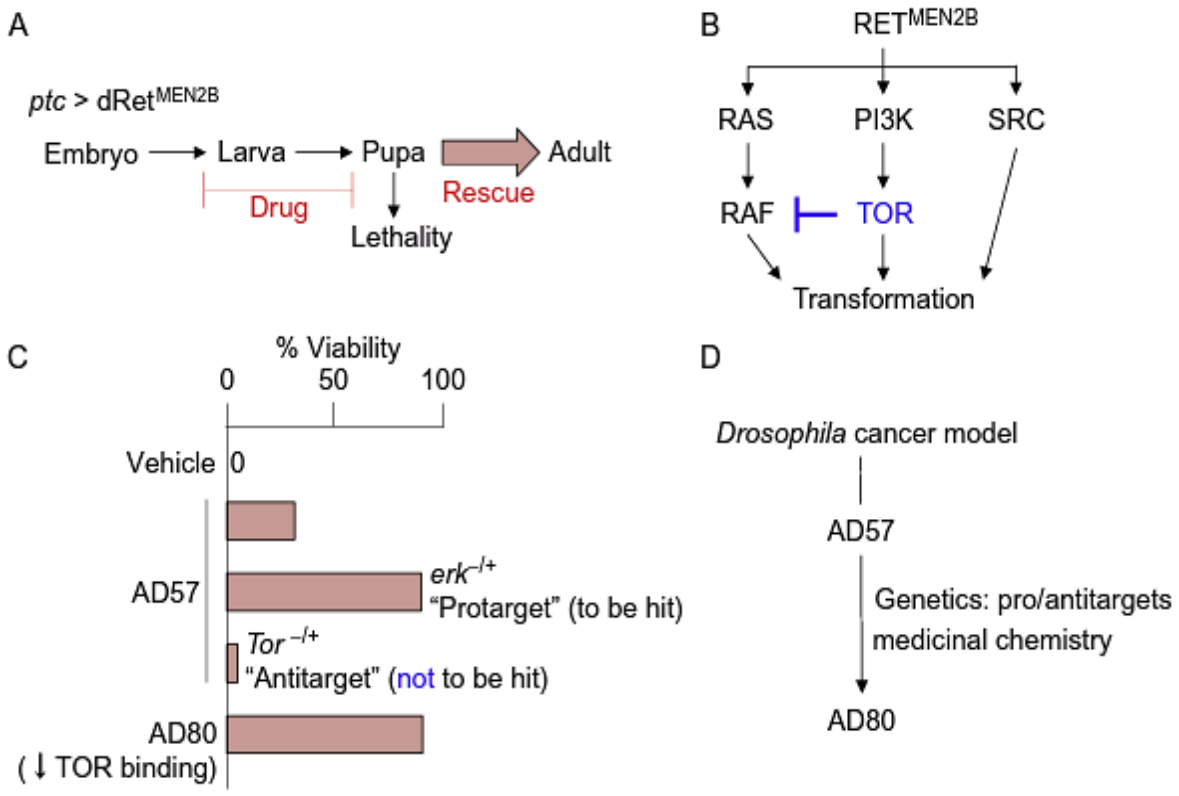

Figure 3. Fruit flies cancer model as a platform for drug development. (Sonoshita and Cagan, 2017)

Additional opportunities for discovery lie in other types of cancers, including those derived from blood cells. Considerable work has been performed indicating conservation in blood cell development between humans and flies, including the study of JAK/STAT signaling (Bina *et al.*, 2010) and lozenge/Runt-related transcription factors (Braun and Woollard, 2009) in hematopoietic cells; however, high-throughput assays for therapeutic discovery pertinent to blood cancers such as leukemia remain to be established, though, there are limitations of *Drosophila melanogaster* in cancer research. While fundamental molecular mechanisms, basic tumorigenesis, and metastasis can probably be efficiently investigated in fruit fly, *D. melanogaster* has not been discovered to model many types of tumors that are common in humans, such as those related to specific tissues (e.g., prostate, ovarian, or breast cancer).

4. 3. Cardiovascular disease

Cardiovascular disease (CD) and associated illnesses are the primary cause of death in the United States, and therefore a greatly required area for the development of novel and more

effective therapeutics. Recent work has indicated that *Drosophila melanogaster* can be used successfully in the discovery process for CD. A key reflection to keep in mind is that CD is for the most part intricate multifactorial disorders that involve heredity as well as environmental factors, and that though certain aspects of CD can be modeled in the fly to yield informative results, the inherently complex nature of the cardiovascular system (CVS) in humans presents certain limitations in the fly for accurate modeling.

For instance, the fly heart has only one cardiac chamber and has no coronary arteries. Fly heart development depends on a set of genes conserved up through mammals (Reim and Frasch, 2010; Bryantsev and Cripps, 2009), and sophisticated tools have been developed, including tomography, to allow its function to be explored in detail (Choma *et al.*, 2006; Null *et al.*, 2008; Bradu *et al.*, 2009). Several forms of dysfunction that include structural defects, cardiomyopathies, and arrhythmias are known to occur in natural populations of flies (Ocorr *et al.*, 2007c). Many of these effects can be age-related and even result in cardiac failure in the fly (Ocorr *et al.*, 2007a, b).

Together, these aspects of the fly's heart and its function indicate that the fly can be an appropriate model for the study of aspects of mammalian CD and a significant tool in the process to discover new therapeutics (Wolf and Rockman, 2008; Wu and Sato, 2008; Akasaka and Ocorr, 2009). Significantly, the beating fly heart can be detected through a traditional dissection microscope for analysis. An excellent resource for protocols on visualization, dissection, and electrophysiological recorded from larva's heart is a publication from Robin Cooper and the accompanying video tutorial (Cooper *et al.*, 2009). Using these methods, it is possible to easily study the effects of pharmacological agents on heart function (Dasari and Cooper, 2006; Dasari *et al.*, 2007; Neckameyer *et al.*, 2007).

Additional tools to facilitate the examination of the heart include GAL4 drivers that can be used to express GFP in the heart, allowing for real-time observation of function with conventional epifluorescence or confocal microscopy (Wu and Sato, 2008; Alayari *et al.*, 2009; Vogler and Ocorr, 2009). Hence where does the fly fit in the total scheme of the discovery process for cardiovascular disease? One vital role is in the discovery of new targets through genetic methods to identify components essential for heart function (Kim *et al.*, 2010; Neely *et al.*, 2010) for which consequent traditional small-molecule discovery can then be performed against. There is also a role in the validation process of positive hits from more traditional screens to assess the actions of particular drugs on cardiac function using low-throughput methods (Akasaka and Ocorr, 2009). According to the recent advances in genetic and imaging tools available to examine fly heart function, it is hoped that higher throughput methods will be soon developed enabling this powerful model to be used for small molecule discovery.

4. 4. Inflammation/ Infectious Disease

Drosophila melanogaster has a very sophisticated immune response that current research demonstrates, and is highly significant to the understanding of human inflammatory conditions. Flies are always exposed to pathogens within their environment, mostly in the form of bacteria, both as larvae and as adults. In response to pathogen challenge, antimicrobial peptides are released through two main pathways that involve evolutionarily conserved components, including Toll and Toll-like receptors, along with, tumor necrosis factor, nuclear factor-B, and JAK/STAT signaling. However, the fly has a sophisticated innate immune system, principally developed to combat bacterial and fungal pathogens, it does not have an adaptive immune

system. Thus, a possibly significant limitation is that the fly is not a suitable model for the study of antibody and lymphocyte-dependent adaptive immune defenses.

Several articles are recommended for more comprehensive reviews of the basic physiology of the inflammatory response and immune signaling in the fly such as; Kauppila *et al.* (2003), Ferrandon *et al.* (2007), Wu and Silverman (2007), and Hetru and Hoffmann (2009). Although there is a possibility that multiple human inflammatory conditions can be modeled and used in the discovery process, the *Drosophila melanogaster* model for asthma, which is the most common chronic inflammatory disease of the lung, is perhaps the most advanced.

The fruit fly respiratory system is the trachea, which entails about 10,000 interconnected and branching tubules. Significantly, there are many conserved genes and regulatory components between trachea development in the fly and lung development in mammals (Liu *et al.*, 2003; Horowitz and Simons, 2008).

Airway epithelial cells from the trachea, and are the first line of defense against airborne pathogens. Unlike mammalian airways, the *Drosophila* trachea is much simpler and consists of only one type of epithelial cell (Whitten, 1957; Horowitz and Simons, 2008). Because there is only one type of epithelial cell, it is a cell culture model within an intact organism, and an immune response initiated from any one part of the tracheal system is identical to that started from another. Inflammatory responses in the trachea to pathogens include Toll, c-Jun NH2-terminal kinase, tumor necrosis factor, and JAK/STAT signaling activity (Wagner *et al.*, 2008). Opportunities thus exist to further develop and utilize the fly in the asthma therapeutic discovery process (Roeder et al., 2009).

One area where *D. melanogaster* shows precise promise is in target discovery. Numerous genetic tools are available, including GAL4 drivers that can drive transgene expression specifically in the trachea (Shiga and Tanaka-Matakatsu, 1996; Liu *et al.*, 2003). One method for exploration is to use these strains to drive small interfering RNA elements in the trachea to selectively knockdown expression of genes whose human homologs are important for airway physiology and the development of asthma to produce an abnormal physiological phenotype (Roeder *et al.*, 2009). Proteins and genes known by modifier screens and forward genetics that release these mutant phenotypes represent starting points for traditional high-throughput small-molecule discovery for drugs that would positively influence the function of not only the fly protein but also the human protein. Another part of target discovery, although more indirect, would be to use the fly as a platform to confirm novel genes and proteins known from next-generation sequencing and human whole-genome association studies (Moffatt *et al.*, 2007) for function in airway epithelial cells and the trachea. Both of these approaches to target finding have the potential to detect and authenticate key components of airway function that represent “druggable” targets for asthma treatments.

4. 5. Metabolic Disorders and Diabetes

Metabolic disorders such as diabetes and obesity are major health problems in the United States. Approximately 4% of the population has diabetes and also, two-thirds of the adult population is overweight. Thus, this has become an attractive area for drug discovery. Though fruit fly has not yet been used in the drug discovery process for this area, recent developments in the understanding of glucose homeostasis, metabolic processes, and endocrinology in the fly have dignified *Drosophila melanogaster* as a valid model significant to human metabolic disorders and diabetes that can be used in the therapeutic discovery field. Although the molecular mechanisms and signaling underlying metabolic processes are conserved to a degree,

a potential restriction of the fly is that the structures mediating these processes are quite different. For instance, the fly does not have a liver or pancreas that secretes insulin. Besides, unlike mammals, it is not possible to feed flies a "Western diet" and have them become obese and eventually develop metabolic syndrome. *Drosophila melanogaster* has neurosecretory cells (Na¨ ssel and Winther, 2010) in the brain that secrete insulin, alongside additional secretory cells that secrete a glucagon analog that together exhibits physiological and genetic parallels to the vertebrate endocrine axis (Wang et al., 2007; Haselton and Fridell, 2010). The removal of the adult insulin-secreting cells can lead to increased glucose levels in the hemolymph (the "blood" of the fly), resistance to starvation, increased circulating lipids, and other phenotypes (Baker and Thummel, 2007; Haselton and Fridell, 2010).

The fat body and the fat cells in fruit fly perform functions related to those of the mammalian liver and are regulated by insulin through mechanisms conserved in mammalian systems in terms of metabolism and triglyceride and glycogen storage (Di-Angelo and Birnbaum, 2009). In correlation to the use of flies as a model to study diabetes, flies express a homolog of the human sulfonylurea receptor that, besides the inward rectifier potassium channel, forms an ATP-sensitive potassium channel to regulate the release of certain fly hormones, including a fly hormone with glucagon-like function (adipokinetic hormone) (Kim and Rulifson, 2004). It is noteworthy that antidiabetic sulfonylurea drugs, including glyburide and tolbutamide, affect glucose homeostasis in the fly through interactions with the ATP-sensitive potassium channel on neurosecretory cells (Nasonkin *et al.*, 1999; Kim and Rulifson, 2004). It is also noteworthy that *D. melanogaster* flies deficient in insulin production demonstrate a delay in development as well as small body size, both as larvae and as adults (Rulifson *et al*., 2002; Kaplan *et al.*, 2008; Ruaud and Thummel, 2008). Body size is a scorable phenotype and may be useful in the development of high throughput screens for the identification of small molecules able to rescue insulin secretion-deficient mutants. Hence, the flies may be used in the discovery, screening, and validation phases for diabetes/metabolic disorder treatments. Therapeutic classes agreeable for discovery may be limited, though. For example, sulfonylurea drugs have efficacy in insulin-deficient flies, but other classes of therapeutics, such as metformin have not been validated to be effective.

4. 6. Spinocerebellar ataxia 3

Spinocerebellar ataxia type 3 (SCA3 or Machado-Joseph disease) is an autosomal, dominant inherited disease initiated by repeats of the CAG trinucleotide at specific gene loci on chromosome 14, that result in polyglutamine portion elongation of the ataxin-3 protein (*Rüb et al.*, 2002). This etiological process is common to all the polyglutamine (poly Q)-related diseases, which also include Huntington's disease, dentatorubral-pallidoluysian (DRPLA), spinal and bulbar muscular atrophy (SBMA), and many types of spinocerebellar ataxia (Dauer *et al.*, 2003). All these disorders are progressive and neurodegenerative. Nevertheless, the pattern of degeneration and therefore the clinical features differ between them (Paulson *et al.*, 2000). 20 Spinocerebellar ataxia type 3 typically presents with dysarthria (slurred speech) and both limb and gait ataxia, weakening over time. 18 Fruit fl*ies* have been used to establish these diseases, the first transgenic *Drosophila* model of SCA3 was created in 1998 (Marsh, 2006). 2 Elongated polyQ proteins (disease proteins similar to that in human disease) 78 residues long were introduced into the flies using the SCA3 gene and these flies were compared with controls expressing proteins with polyQ runs of only 27 residues.

Features of SCA3 were revealed in these diseased flies, allowing for this model organism to be used in advance research into the neuronal loss and mechanisms of degeneration in SCA3 (Paulson *et al.*, 2000). 20

4. 7. Huntington's disease

Huntington's disease is another polyglutamine (PolyQ)-a related disease that has been studied using *Drosophila melanogaster*. It is a neurodegenerative disease that has an autosomal dominant inheritance and is clinically characterized by choreic movements that worsen over time, accompanied by psychiatric disturbances and cognitive decline. It is currently understood to be caused by abnormal polyQ expansion at the N-terminus of the huntingtin protein. Expansion beyond 36 repeats results in the neurotoxic huntingtin protein found in Huntington's disease. 21 Homologues of the Huntingtin protein have been found in other vertebrates including Mice, Pufferfish, and Zebrafish, but because of the similarity in amino acid sequence, functional domains of the protein could not be easily distinguished. Studies using the more distantly related *Drosophila* have allowed for the identification of conserved protein domains that are likely to be significant in the function of the Huntingtin protein (Li *et al.*, 1999). Further work using *Drosophila* has led to conclusions that while the Huntingtin protein is normally contained in the cytoplasm, mutated forms are localized to the nucleus. Additionally, inclusions, which are large aggregates of the mutated protein and transcriptional co-activators, are found in neurons contributing to the pathology of this disease (Marsh *et al.*, 2003).

The use *of Drosophila melanogaster* does not only provide insight into the cause and pathogenesis of Huntington's disease, but it also provides prospects for research into therapeutic intervention. Agrawal *et al.* (2005) proposed and studied the efficacy of combination drug treatments in the management of Huntington's disease; through their work with the fruit fly, they identified two combination treatments as potential candidates 24. Agrawal *et al.* (2005) introduced mutant human huntingtin protein into *Drosophila* and analyzed prevalent neurodegeneration through climbing assays, survival assays, and pseudo pupil assays. They used drug-feeding experiments to assess the effects of combination drug treatments on disease progression and established that the two treatments tested (Congo red, cystamine, and SAHA or SAHA combined with geldanamycin or Y-27632) inhibited neurodegeneration and therefore may be effective in alleviating symptoms in people with Huntington's disease.

4. 8. Alzheimer's disease

Alzheimer's disease has become an ever-increasing problem for people, especially with the aging population of the Western world. Additional research is thus needed to help us in comprehending the disease and how to develop interventions in the future. *Drosophila melanogaster* is a perfect model organism to describe Alzheimer's disease as it can be made to show the signs of the progressive neuronal degeneration seen in this disease.

The pathology consists of the formation of β-amyloid plaques composed of neurofibrillary tangles and amyloid β1-42 formed from hyperphosphorylated tau protein. Through genetic modification methods, such as the Gal4/UAS system, we can make *Drosophila* manufacture amyloid β1–42 as well as amyloid plaques, which make the fly mimic Alzheimer's disease in humans. The Gal4/UAS system can also be used to introduce R406W, the tauopathy-associated mutant of human tau, into *Drosophila* to reproduce neurofibrillary tangles

analogously. Evaluation of these transgenic flies via longevity assays, locomotor and climbing assays, and olfactory learning assays allows for further assessment of how Alzheimer's disease affects humans. The progressive locomotor decline can be observed in transgenic *Drosophila* through climbing assays. Additionally, the crawling velocity of larvae can be measured to evaluate the effects of tau phosphorylation on motor neurons. Pavlovian olfactory learning assays, in which the *Drosophila* linked a certain odor with an electric shock and therefore learn to move towards the control odor in a T-maze, have shown reduced learning in flies containing Aβ1–42. Histological assays establish the changes that occur at the neuronal level at different stages as the disease progresses. The use of *Drosophila melanogaster* in Alzheimer's disease research allows more understanding and potential discovery of new pathological processes and identification of new molecular targets for drug development (Crowther *et al.*, 2006).

4. 9. Parkinson's disease

Parkinson's disease (PD) is another progressive neurodegenerative disease for which fruit flies have been a key in research and pathological development. It is a chronic, progressive neurodegenerative disease with global distribution and increasing occurrence with age (Thomas *et al.*, 2007). An English apothecary and surgeon, James Parkinson (1755–1844), was the first to describe this disease in his 1817 article titled, *Essay of the Shaking Palsy'* (Parkinson, 2002), in which he drew the core clinical manifestations (Dauer *et al.*, 2003) of this physically and socially devastating disease (Thomas *et al.*, 2007). These include postural instability, resting tremor, bradykinesia, gait difficulties, and muscular rigidity. The pathology of PD begins from progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) (Wood-Kaczmar *et al.*, 2006). The majority of cases of PD are periodic, with unknown causes. The environmental postulate suggests that these cases result from exposure to dopaminergic neurotoxins in the environment such as paraquat and rotenone, used as an herbicide and an insecticide respectively. Both are structurally similar to MPP+ (Neurotoxin), the active metabolite of MPTP, which can cause mitochondrial defects (Dauer *et al.*, 2003).

Though, 5–10% of Parkinson's disease cases appear to have familial etiology linked to the following genes: DJ-1, α-synuclein, Parkin, LRRK2, and PINK1, they have been identified to have both recessive and dominant inheritance. Understanding these genetic components has led to more awareness of the molecular pathogenesis of PD (Thomas *et al.*, 2007). *D. melanogaster* is being used today in the study of these hereditary causes of PD. One of the genes linked to familial PD, α-synuclein, is not found in *Drosophila*. This gene encodes a protein which is a component of Lewy bodies that are implicated in the pathology of PD (Park *et al.*, 2009). Both Feany and Bender introduced human α-synuclein into *Drosophila* by the use of the GAL4/UAS system to establish neurodegeneration, the formation of inclusions, and resultant defects in locomotion caused by α-synuclein toxicity (Feany and Bender, 2000) 31. They used tyrosine hydroxylase staining to analyze the dopaminergic neurons in sections of the fruit fly brain, looking directly for neurodegeneration and used climbing assays to analyze the relating locomotor response (Feany and Bender, 2000). Climbing assays were also used by Greene *et al.* (2004) in their study of PD, mutations in which were already known to cause a form of early-onset Parkinson's disease called autosomal recessive juvenile parkinsonism (AR-JP) Parkinson's disease (Feany and Bender, 2000).

They studied the longevity, flight, and climbing abilities of *Drosophila* containing mutant PD. Results confirmed reduced permanency and defects in flight and climbing, as well as male sterility. As changes in the structure of mitochondria are a common feature of both muscle and

germline pathology, they concluded that mitochondrial dysfunction must be important in the mechanism of dopaminergic neuronal loss in PD (Greene *et al.*, 2003).

5. CONCLUSION

Drosophila melanogaster has been of importance to the study of human diseases for over 100 years. Further understanding into neurodevelopment and the function of the nervous system has been influenced significantly by work done in *Drosophila melanogaster*, which leads to the research carried out today on neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. In this review, we highlighted amusing areas for future work in *Drosophila* infection and immunity, we also discussed new potentials for translation of this work into the realm of human medicine. With the aging population of the Western World, further research into this research area will be greatly beneficial.

Acknowledgement

The authors are grateful to Mr Ochala and Mr Akachukwu Obialor, Drosophila Laboratory, Africa Center of Excellence in Phytomedicine Research and Development, University of Jos, for providing us with basic knowledge on Drosophila which prompt us to write of this manuscript

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