Analysis of Comparative Efforts between the *Drosophila melanogaster*'s Genome and the Human Genome

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Abstract: *Drosophila melanogaster* (*D. melanogaster*) has been used in biomedical research for over a century. Studies have included the study of genetics and inheritance, embryonic development, learning, behavior, ageing, drug discovery, and evolution. The reason for its centrality in those diverse fields is the fact that *D. melanogaster* shares many homologous genes with other species, including humans. In fact, Pandey and Nicholas (2011)[13] state that "nearly 75% of human disease-causing genes are believed to have a functional homologue in the fly". This resemblance proves that comparison is an essential part of the biomedical field, as the *D. melanogaster* is still considered a great model organism, allowing scientists to study the impact of mutations on the fly, which can also form inferences that impact the welfare of humans. Despite the numerous studies done on the fruit fly, surveying the available literature has shown several vital pieces of information that are yet to be picked up for future research. The fields of study are extremely diverse, and include studies on the dopaminergic neurones, the function of specific exons, Alzheimer's Disease, and pathogenic viruses. This paper aims to shed light on the discoveries and advances done in recent research in order to help direct the progress of future studies concerning the *D. melanogaster* in studying viruses, neurodegenerative diseases, and improving existing pharmaceuticals, as these are the fields where most of the studies in recent years have been conducted. This guidance is done by compiling and synthesising the existing literature, and presenting the recommendations put forth by the aforementioned studies.
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Comparative Efforts Using *Drosophila melanogaster*

Drosophila melanogaster's usage in biomedical labs has been traced back by Kohler to 1901 by William Castle[1]. It led to several discoveries that were worthy of the Nobel Prize. These discoveries include evidence supporting that DNA as the genetic material[2], proving that X-rays cause mutation by physically breaking chromosomes[3], and understanding how genes direct the development of embryos to mature multicellular organisms[4].*D. melanogaster*has also been used to study inheritance, learning, behaviour, ageing, and clinical drugs[5,6]. Evolution of population genetics[7], proteins[8], and DNA sequence levels[9], has also been heavily studied, which has led to a great level of understanding to the process of molecular evolution[10]. In fact, the majority of the current knowledge regarding cellular, tissue, and regenerative biology comes from studying model organisms such as the fruit fly[11].

Several factors have caused *D. melanogaster* to be considered a model organism in biomedical labs. The fruit fly shares many homologous genes with humans[12], and it is believed that around 75% of human disease-causing genes have a functional homologue in the fly[13]. These evolutionary conserved genetic sequences have been exploited by researchers to study the function of several genes[14].

Having relatively simple genetics with reduced genetic redundancy, in addition to the ease involved in manipulating its genes, *D. melanogaster* is an excellent model in studies pertaining to molecular replication, amplification, and cellular consequences of human viruses[15]. This utility is also further reinforced once the number of potential progeny from each female fly is considered, and the short amount of time the eggs require to hatch[16,17]. Using model organisms such as the fruit fly also reduces the ethical and practical obstacles faced when conducting experiments in human biomedical science. The fruit fly's rapid lifecycle also means that less time would be needed to conduct studies, especially when compared to the vertebrate models such as mice and zebrafish[11].

Difficulties are still present when using *D. melanogaster* as the target of genetic research, and these difficulties include the pleiotropic nature of genes[18], and the fact that the homologous genes in the fruit fly do not mimic the phenotypes found in humans[19]. There are also physiological differences between *D. melanogaster* and humans, such as the optimal body temperature, and the absence of some human genes in the fruit fly's genome. There also

exists some biochemical differences with respect to cellular surfaces[19], and the presence of neurotransmitters[20]. Even with the existence of these differences, studies on the *D. melanogaster* will continue to provide insight with respect to the pathophysiology of several diseases[21]. Moreover, these differences can be overcome through the modification of the *Drosophila*'s genome or cellular structure[22,23].

Common Methods Using *Drosophila melanogaster*

The "Binary GAL4/UAS Gene Expression System" has shown great success in introducing genes to the *D. melanogaster*'s genome. The gene of interest is synthesised in a way that would place it under the regulation of the "Upstream Activating Sequence"(UAS), which is activated by binding to the GAL4 transcription factor. A transfected fly would be crossed with a fly containing the GAL4 driver, which produces progeny containing both properties .This method has proven to be quite useful in the determination of gene function, which could be over 5kb in size. Owing to GAL4's sensitivity to temperature, gene expression could be controlled[24]. More transgene copies could also control gene expression[25], in addition to changing vectors[26].

Viruses and *Drosophila melanogaster*

For over a decade, the fruit fly has been employed in research regarding the molecular and genetic functions of pathogenic viruses, while also giving crucial insight into host antiviral immunity[27]. Different methods have been employed to study different viruses. The infection of *D. melanogaster*'s cells was used for the Dengue Virus[28,29], Influenza A Virus[30], and Sindbis Virus[31]. Transgenic lines were formed to study the Epstein-Barr Virus[32], Human Immunodeficiency Virus[33,34], Human Cytomegalovirus Virus[35], Influenza A Virus[36], Severe Acute Respiratory Syndrome Coronavirus[37,38], and Simian Vacuolating Virus[39]. *D. melanogaster* cell cultured were also transfected to study the Hepatits B Virus[40], and Human Immunodeficiency Virus[41].

Severe Acute Respiratory Syndrome Corona Virus

The SARS-CoV (Severe Acute Respiratory Syndrome Corona Virus) was the causative agent involved in the pneumonia epidemic in 2003[42]. The introduction of its 3a protein to the *D. melanogaster* caused an increase of apoptosis in the developing eye[43]. This phenomenon is believed to be mediated by cytochrome c in the mitochondrial pathway, which is identical to its effect in human cells[44]. Effects on other cellular processes have also been linked to the 3a protein, including calcium ion regulation, ubiquitination, and transcription. Pharmaceutical intervention to block the ion channel involved in transporting the 3a protein has been shown to prevent its effects in human cells (*in vitro*) and the transgenic flies (*in vivo*). Another protein, the M membrane protein, has been found to induce eye apoptosis by suppressing survival signalling pathways[37]. Identifying ways by which the actions of 3a and M membrane proteins can be inhibited could potentially lead to the alleviation of symptoms[15].

Human Immunodeficiency Virus

The HIV (Human Immunodeficiency Virus) causes around 1.5 million deaths annually[45]. Its pathogenicity arises from its reverse transcriptase activity, which allows its DNA to become permanently integrated into the host cell's DNA[46]. The HIV-Nef membrane-associated protein was studied using the *D. melanogaster*, where it proved to cause downregulation of the cell surface receptor of CD4 cells through endocytosis[22]. This study was done by transfecting *Drosophila* Schneider 2 (S2) cells with human CD4 protein and the HIV-Nef genes, which resulted in their co-expression. The endocytosis was found to be mediated by clathrin. The effects on the flies include larval wing disc apoptosis, and the inhibition of the NG-kB signalling in body cells, which corresponds to a decline in T-cell immune function in humans[47]. That is another protein that is essential for viral replication. It elicits its action by disrupting microtubule polymerisation and kinetochore dynamics in the *Drosophila*[33], which corresponds to an inhibition in rRNA processes in humans, leading to a reduced number of ribosomes in the cytoplasm[48]. The HIV-Rev protein was found to regulate expression of the HIV genes, but more studies are required on it as its mechanism of action remains unknown[15].

Neurodegenerative Diseases and *Drosophila melanogaster*

Neurodegenerative diseases (NDs) is a term that describes a diverse group of diseases that are characterised by a progressive worsening of neural functions including loss of sensation, motor control, memory, and cognitive impairment. Manipulating multicellular organisms that possess a nervous system can help provide insights on the cellular and molecular mechanisms of these diseases, which could result in methods to delay, or cure the symptoms caused by NDs[19,49,50]. This manipulation is possible because the genes involved in NDs are evolutionary conserved in higher eukaryotes[19]. One of the key aspects of NDs is their association with mitochondrial dysfunction which causes an accumulation of reactive oxygen species (ROS)[51-53]. Mitochondrial respiratory complexes I and III produce ROS as a byproduct of their reactions, so defects in these complexes can result in an excess of ROS[19]. Another biochemical explanation involves misregulation of dopamine (DA)[54]. Since DA synthesis and secretion mechanism

pathways, in addition to receptors and transporters, have been conserved between flies and humans, *D. melanogaster* serves as a suitable model organism to study the abnormalities seen in NDs[55]. It should be noted, however, that DA metabolism pathways are different[21]. The genes essential for melanin synthesis in the insect cuticle, which is derived from DA's precursor tyrosine in both mammals and insects[56], also regulate DA synthesis in mammalian and insect brains[57,58].

Parkinson's Disease

Parkinson's disease is a ND that has been studied extensively in *D. melanogaster*[59-62]. It was previously proven that increased DA levels cause the "removal of memories" or "forgetting" in flies[63]. This removal of memories is similar to dementia, which is characteristic to Parkinson's disease in humans; the correlation between these two observations can lead to an improvement to existing therapies[21]. It was also proven that introducing MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a chemical that inhibits mitochondrial complex I, results in Parkinsonism[64], which is homologous to the effect of using the insecticide rotenone and the herbicide paraquat on insects[65].

Alzheimer's Disease

Alzheimer's disease is another ND where *D. melanogaster* was found to be useful[66]. The first indication that the disease had a genetic component was due to the observation that first degree relatives are more likely to develop the disease[67,68]. Certain proteins have been identified which have been attributed as the cause for early-onset Alzheimer's Disease, and these include Amyloid Precursor Protein (APP), Presenilin 1, and Presenilin 2[69]. The disease is characterised by beta amyloid plaques and tau proteins in the hippocampus[70]. When these proteins were overexpressed in the *D. melanogaster*'s retina, the result was flies with tough and smaller eyes[66,71]. While APP in humans has App1 as a homolog in flies, the product of its cleavage was not evolutionary conserved[72]. However, the product of App1 cleavage in flies produced Aβ42, which is the main constituent of the amyloid plaque in *D. melanogaster*[73], and has been attributed to cause a reduced lifespan in flies, in addition to brain and photoreceptor degeneration, and impaired locomotion[74,75]. Other proteins that were connected to Alzheimer's disease in humans are Apolipoproteins D (ApoD)[76] and E (ApoE)[77]. ApoD has Glial Lazarillo (GLaz) as a fly homolog, while ApoE does not seem to have one. A mutation causing reduced GLaz expression produced flies with reduced resistance to oxidative stress and starvation, in addition to impaired fat storage and a shortened male fly lifespan[78]. An overexpression of GLaz (and human ApoD) produced flies with an increased resistance to hyperoxia and starvation, in addition to extended lifespans[79-81]. This overexpression led to the conclusion that they have protective roles in stressful conditions, and their reduced expression results in a faster rate of neurodegeneration[78,81].

Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's Disease, is used to describe a condition in which the upper (cortical) and lower (spinal cord) motor neurones degenerate progressively causing muscular dysfunction, and eventually paralysis[82]. Most of the cases have no positive family history or a genetic cause[82]. Several mutations have been attributed to the pathogenesis of ALS[19], which includes Copper/Zinc Superoxide Dismutase (Cu/Zn SOD)[83]. The expression of human SOD1 in *D. melanogaster* resulted in an extended lifespan when compared to normal flies[84], which is potentially attributed to an augmented ROS metabolism and resistance to oxidative stress[85].

Vesicle-Associated Membrane Protein (VAMP)/Synaptobrevin-Associated Membrane Protein B (VAPB/ASL8) is another protein complex that was associated with ALS[77]. Flies with mutations in VAPB display filamentous and enlarged mitochondria in muscle cells[86], which has been attributed to a reduced mitochondrial ability to properly buffer calcium ions[87], leading to spikes in calcium ions and spontaneous muscular contractions, akin to those observed in ALS patients[88,89]. This mutation also corresponds to a decreased level of Bone-Morphogenic Proteins (BMP) in humans, which has a homolog named Gbb in flies[90]. A reduced level of Gbb in flies leads to less pMAD (a signalling pathway) in the presynaptic terminal[91], which in turn leads to a loss of neuromuscular junction maintenance[92]. This loss of maintenance is similar to the observations made in SOD mice and ALS patients[93,94]. The end result is synaptic retraction and loss of motor neurons in sporadic ALS[95], SOD[96], and ALS8[97] patients. Research focusing on the role of BMP and its external administration to ALS patients may be beneficial[19].

Polyglutamine Diseases

Polyglutamine (PolyQ) Diseases is a collective name given to a total of nine diseases caused by "CAG" repeats in the translated regions of unrelated genes[19]. The overexpression of the PolyQ expanded proteins in the retina of *D. melanogaster* resulted in rough, depigmented eyes. This phenotype allowed the genes to be exhaustively reviewed in flies[98]. One important PolyQ disease is Huntington Disease, which is caused by the expansion of a CAG triplet in the "huntington protein" (HTT). The introduction of this protein into the fruit fly resulted in a pathology. In order to determine the most pathogenic portion of

HTT, its exons were separated and introduced into flies to produce transgenic flies. In general, the formation of huntingon aggregates resulted in a progressive loss of motor function in flies[99]. The aggregates seem to elicit their action by impairing calcium ion buffering and excitotoxicity, which could be the main cause of neurodegeneration[100]. The results also proved that exon 1 of the HTT peptide was relatively the most toxic and pathogenic. Future research can be directed at finding methods to reduce its toxicity, and by extension its pathogenicity[99]. It was also found that reducing oxidative stress on *D. melanogaster* has no impact on polyQ diseases[101] while enhancing levels of NADPH through the overexpression of the HSP27 gene[102,103] increased the flies' lifespan, which could be due to the role of NADPH in reducing toxicity induced by polyQ diseases[104].

Drugs and *Drosophila melanogaster*

A plethora of studies exist which prove that the *D. melanogaster* is a practical model for studying drugs. It has been used in the discovery process[5,6], in addition to screening for potential drugs[104]. After identifying the pathogenesis of certain disease-causing mutations, drugs can be fed to the flies in order to determine ways to inhibit the mutant phenotype[15]. Studies have proven that drugs affecting the metabolism of dopamine have an effect on flies[13,48,49]. Several have studied the effects of Reserpine, a human antipsychotic drug, and its mechanism of inhibiting dopamine signalling*in vivo*[105-107]. Focus was placed on dopamine is because it is involved in the reward-signal pathway in human brains, which has been associated to several addictions[21]. In fact, drugs for Parkinson's disease that focus on increasing the levels of L-Dopa in the brain have been noted to cause an increase in hedonistic behaviour[55]. Whilethe insect analog is octopamine[108], the suppression of dopamine levels in *D. melanogaster* led to a reduction of the locomotion impairment elicited through the intake of ethanol, nicotine, or cocaine[105]. This proved the continued viability of the *D. melanogaster* as a model organism in studying drugs.[109].

D. melanogaster cell cultures can also be used to produce drugs, specifically human monoclonal antibodies[110]. While bacteria and fungi are the normal candidates[111], bacteria has the drawback of not being able to produce correctly-folded glycosylated antibodies[112], and yeast was only successful in producing them in minimal amounts[113]. With the prediction of an increased demand for monoclonal antibodies, more efficient methods of production are required[114,115]. *Drosophila* Schneider (S2) cell lines have proven to be a lot more productive, in addition to being more stable and consistent in their growth profile, protein production, and are still functional after months of storage[110,116-118]. They can grow in Serum Free Media, and the cost of raw materials and consumables involved is almost seven times less expensive that the commonly used methods[119,120]. By using Wave Bioreactors to culture transfected S2 cells, the concentration of the produced human monoclonal antibodies was 28 times higher than when other organisms were used[110].

Miscellaneous Studies Performed on *Drosophila melanogaster*

D. melanogaster has been used to study cocaine tolerance and withdrawal research[18,121,122], identify exact functions of human proteins[123], uncover essential factors for successful fertilisation and zygote formation in humans[124], and recognise the role of certain neurotransmitters in aggression[125] and sexual orientation[126]. Studies on Down's syndrome[127]and Alzheimer's disease[128] have been related to the misexpression of the gene DSCR1, which is homologous to the *sarah (sra)* gene in flies. The role of DSCR1 in humans remains unclear[129], while the role of sra in *D. melanogaster* females is associated with reduced receptivity to males[130].

Conclusion

With comparison being its key aspect, many advances have been done based on *D. melanogaster* to improve human life and regenerative medicine[15]. It is expected that it will continue to be crucial in the future[26]. By synthesising the most recent information in one literature review, this paper attempts to guide future studies. This guidance will hopefully result in a better understanding of current diseases, and the discovery of ways by which the quality of human life could be improved. Certain limitations exist in this approach, including the difficulties involved in accessing the most recent and unpublished papers, in addition to overlooking older works.

In order to add to current knowledge, several areas of research still exist. Further studies to determine the functions of each exon in the *D. melanogaster's* genome are needed[131]. Areas of study include identifying the role of dopamine in human sleep disorders such as hypersonmia, REM (Rapid Eye Movement) sleep behaviour disorder, and restless legs syndrome[21].

With respect to Alzheimer's disease, there is still a lack of knowledge regarding the effects of Aβ42 and tau toxicity in the *D. melanogaster*[19]. After isolating the most toxic exon involved in the HTT peptide of Huntington's disease, more studies should focus on ways that can reduce its pathogenicity[99]. Determining the role BMP plays and its external administration to patients with Amyotrophic Lateral Sclerosis (ALS) may be crucial for the development of future therapies[19]. More data is required to exploit the

resistance to ROS-related oxidative stress observed in flies with the expression of the human SOD1 gene[19]. Additionally, more viruses can be studied using the *D. melanogaster*, and these include the Human Papillomavirus (HPV), Hepatitis C Virus (HCV), and the Yellow Fever Virus[15].

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