5-5-2020

Identifying Fluctuating Asymmetry and Developmental Rate as Indicators of Developmental Stability in Drosophila Melanogaster

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Carrie N. Reaver

Senior Honor's Research 2019-2020
Faculty Advisor: Dr. Bjoern Ludwar
Longwood University
May 5, 2020

This thesis has been read and approved by:

Committee Members:

Dr. Brandon Jackson (Longwood University) _____

Dr. Benjamin Campbell (Longwood University) _____

Dr. Ruth Holliday (Longwood University) _____

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1. Abstract

Developmental stability is the ability of an individual to maintain proper development despite various environmental conditions, and thus has important implications for the health of an individual. Individuals with less stable development are thought to be at higher risk for developing non-communicable disease, such as diabetes mellitus and Alzheimer's disease, during adolescence and adulthood. In bilateral organisms, developmental stability can be assessed by measuring deviations from perfect symmetry between the left and right sides of the body, known as Fluctuating Asymmetry. In this project, we measured the developmental rate and Fluctuating Asymmetry of *Drosophila melanogaster* while subjected to varying metabolic conditions differing by sucrose content. Analysis of Fluctuating Asymmetry observed between drosophila populations subjected to different concentrations of sucrose did not yield significant results; however, various trends in the data elucidated the interactions between developmental stability, metabolic stress, and developmental rate. By working to further establish a link between these factors, this project supports the development of cost-effective early screening methods for diabetes mellitus and related diseases.

2. Introduction

2.1. Chronic Disease and Developmental Stability

Chronic diseases reign as a leading cause of death and disability in the United States and account for trillions of dollars in health care costs each year (CDC, 2019). Despite ample clinical knowledge concerning the pathophysiology of most chronic diseases, management and treatment of these diseases remains insufficient. This is due to the fact that many chronic diseases begin to wreak havoc on the body's systems long before any symptoms appear. Additionally, the manifestation of disease varies by individual, making chronic diseases difficult to predict on a case-by- case basis. As a result, detection of chronic diseases often occurs years after disease progression has already begun. While genetic predisposition and lifestyle are two major drivers of chronic disease, the predictive power of these two characteristics still proves inconsistent in early diagnosis of disease. There are ample examples of individuals who strive to live healthy lives and still develop chronic disease, or examples of individuals who have genetic markers for a specific disease yet never develop it. Because of such discrepancies in the behavior of chronic diseases, the clinical approach to detecting and treating chronic diseases remains limited, and thus chronic diseases continue to cause millions of deaths per year in the U.S. alone (CDC, 2019). As rates of chronic diseases such as diabetes - the fast growing noncommunicable disease in the United States (see figure 1) - continue to rise, the need for novel disease detection and management is a national health priority (CDC, 2018).

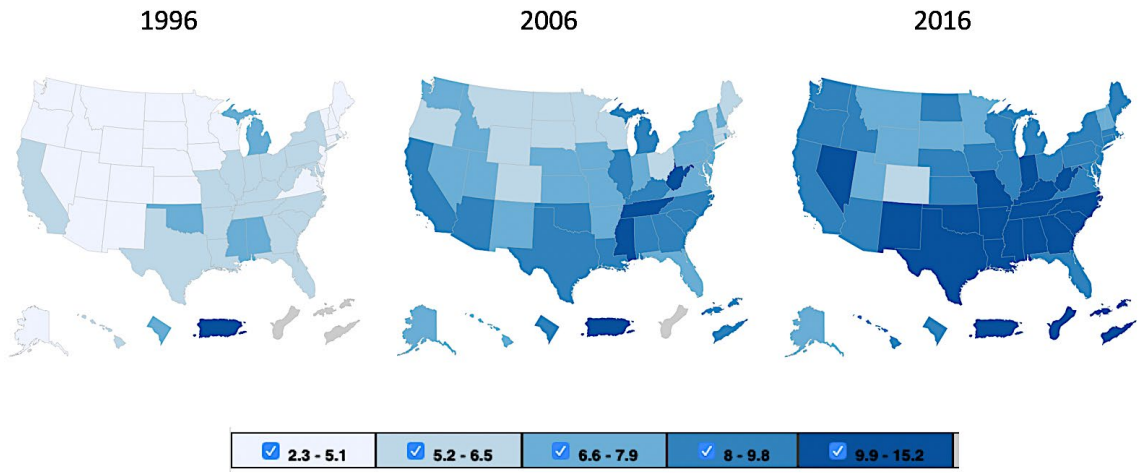

Figure 1.: Age-adjusted percentage of adults diagnosed with diabetes.
Source CDC (CDC, 2018).

Although genetic and environmental factors have so far proved inadequate as early detection criteria, it is well established that these two factors interact in a complex manner to establish one's likelihood of having a disease. Developmental stability serves as a link between these two factors and may be the driving force behind chronic disease development. Developmental stability is the capability of an organism to develop and grow according to a predetermined

genotype despite external disturbances (Waddington, 1959). Essentially, every organism begins life with an established genotype, and development attempts to follow this genetic formula perfectly; however, external disturbances such as acquired genetic mutations or environmental stressors lead to imperfect development. Such imperfections may prove harmless or they could produce a phenotype more susceptible to ailments like chronic disease. Because of this, developmental stability is likely a strong indicator of one's overall health.

Developmental stability has already been implicated in a variety of chronic diseases such as cardiovascular disease, diabetes mellitus, and various cancer types. Recent research in the field of developmental origins of health and disease (DOHaD) has supported the conclusion that developmental plasticity – the ability to adjust genetic expression to produce the most apt phenotype in response to environmental signals during early life – is responsible for establishing a set phenotype for the rest of an individual's life (Lea et al., 2017). Developmental plasticity is an evolutionary tool adopted by organisms to "predict" which traits will provide the most successful phenotype in a postnatal environmental. However, if the individual encounters signals during development that do not correspond to the environment it will encounter after birth, the developed phenotype is oftentimes susceptible to disease or disability. Developmental plasticity is driven by the epigenome, the molecular infrastructure that controls gene expression in response to external signals (Lea et al., 2017). Identical twins, for example, have the same genome yet can exhibit noticeably different phenotypes depending on how each twin's epigenome modulated gene expression in response to different early-life environmental signals. Developmental plasticity thus drives development and sets the stage for the life-long health of an individual during just the first few years of life.

A classic example of the role of development plasticity in disease establishment is the increased rate of cardiovascular disease and metabolic disorder in individuals whose development occurred during the Dutch Hunger Winter (Shultz, 2010). While under Nazi occupation in the winter of 1944-1945, the Netherlands experienced a large-scale famine that left many eating tulip bulbs just to get through the winter, resulting in many pregnant women and children becoming severely malnourished. Interestingly, individuals born during or soon after the famine experienced significantly increased rates of metabolic diseases such as obesity and diabetes later in life compared to the general population. As developing fetuses and young children encountered famine, their metabolisms adjusted for life-long nutrient scarcity. Yet later in life, when these individuals could enjoy a calorically-rich diet, their metabolisms were unprepared, thus resulting in metabolic syndrome. Epidemiologist Dr. David Barker first observed this trend and developed the Barker Hypothesis which states that adverse nutrient supply during development increases predisposition to metabolic syndrome (Barker, 1992).

After the popularization of the Barker Hypothesis, research in the field of DOHaD exploded as researchers searched for more trends between developmental stability, epigenomics, and noncommunicable diseases. Recent studies have elucidated the epigenomic mechanisms that direct cellular physiologies correlating to a specific disease, such as microRNA-driven insulin biosynthesis in type 2 diabetes or mitochondrial DNA methylation in Alzheimer's disease (Esguerra et al., 2018; Qazi et al., 2018). Epigenetic programming is even influenced by the establishment of the gastrointestinal microbiome during early childhood, and several studies have implicated gut microbiota with inflammatory diseases and mental disorders as reviewed by Alam et al. (2017). Undoubtedly, developmental stability and plasticity have tremendous roles in determining the phenotypic characteristics that drive disease susceptibility for the entirety of

one's life, and it is evident that plasticity is heavily influenced by the metabolic environment present during development.

While it is important to acknowledge the immense progress the scientific community has made in understanding the pathophysiology of chronic disease, it is equally important to recognize the limitations that still exist in the early diagnosis and treatment of chronic disease. Despite being able to identify exactly which genes and epigenetic processes may drive the progression of a disease, chronic disease detection using this knowledge is still inconsistent and is often not the complete story behind disease development. To say the interactions between genomics and environment during development are complex is an understatement, and it is practically impossible to compound all the different factors contributing to developmental stability in order to predict the disease susceptibility, as many are still unknown. Luckily, developmental stability evaluates these factors for us and the "result" is reflected in measurable phenotypic characteristics, particularly in bilateral animals.

2.2. Fluctuating Bilateral Asymmetry as a Biomarker of Developmental Stability

In bilateral animals, the genome that dictates one's growth is the exact same for the left- and right-side extremities and thus ideal development would produce an organism with perfect external bilateral symmetry. Likewise, improper development (i.e. the inability of an organism to buffer external disturbances, or developmental instability) would result in small, random variations during growth, resulting in some degree of bilateral asymmetry. Therefore, this random deviation from bilateral symmetry, known as Fluctuating Asymmetry, is a physical phenotypic manifestation of an individual's genetic make-up, history of environmental influences, and overall stability during development (Møller, 1997). Thus, measuring the Fluctuating Asymmetry of an organism's phenotype would be indicative of its developmental stability and ultimately could serve as a predictive variable for early screening methods to detect chronic diseases.

In a recent clinical study, Morris et al. (2016) demonstrated a correlation between diabetes mellitus and the degree of Fluctuating Asymmetry present between fingerprint pairs. Further studies by the same research group indicated that fingerprint patterns, a phenotypic trait that does not change after birth, on certain finger pairs had a relatively high predicative score for type 1 and type 2 diabetes mellitus compared to traditional predicative models such as family history or waist circumference. Measuring fingerprint pairs could therefore be used as an effective, low cost early screening method for diabetes mellitus that could be employed as early as infancy to predict an individual's disease risk. The current project aimed to further investigate the correlation between Fluctuating Asymmetry and diabetes mellitus through the use of a *Drosophila melanogaster* model system subjected to varying metabolic environments during development.

2.3. Modeling Metabolically-Induced Developmental Instability in Drosophila melanogaster

Interestingly, human and drosophila metabolisms are remarkably similar and therefore drosophila serve as useful models for studying metabolic disorders. Despite having different insulin-like peptides than humans, drosophila models of diabetes are widely used and, because one insulin-like peptide in drosophila, dilp8, has been implicated in both metabolism and

controlling body symmetry, drosophila models are ideal for studying the interactions between metabolic environment and asymmetries (Álvarez-Rendón at al., 2018; Colombiani et al., 2015). With the discovery of the connection between fingerprint pair symmetry and diabetes mellitus by Morris et al. (2016), the current project worked to elucidate some of the underlying factors behind this phenotypic manifestation of disease by modeling diabetes in *Drosophila melanogaster.*

In preliminary experiments, drosophila were raised on media with varying sucrose concentrations and then assessed for asymmetry in wing-vein lengths. Surprisingly, these experiments demonstrated that drosophila raised on high-sugar media displayed wing asymmetry to a lesser extent than drosophila raised on media with lower sugar concentrations, contradicting our expectations. Originally, it was hypothesized that drosophila raised on high-sugar mediums would exhibit a higher degree of wing asymmetry compared to drosophila raised on low-sugar. Using a well-established recipe for inducing diabetes in drosophila, we expected to observe more malformities in our high-sugar group as these drosophila were presumed to be enduring the most metabolic stress during development (Musselman et al., 2018). However, another observation made during these preliminary experiments supported a possible alterative hypothesis: the high-sugar drosophila grew at a much slower rate compared to low-sugar flies, indicating that developmental rate could be the influential factor behind the observed asymmetry. Thus, the question remained as to whether developmental stability – and consequently Fluctuating Asymmetry - of our drosophila was driven by early-life nutrient supply or if it was driven by developmental rate.

Research concerning developmental stability of the swordtail fish *Xiphophorus multilineatus* has illuminated the relationship between nutrient supply and developmental rate. Morris et al (2012) observed that male sword fish grew at a faster rate when raised on a high quality diet, resulting in increased Fluctuating Asymmetry of vertical bars, a sexually selected phenotypic trait. When nutrients are favorable during development, an organism may optimize a faster growth rate over developmental stability since the environmental provides less nutritional stressors, requiring a less "prepared" phenotype. Conversely, a high sugar environment may slow growth as an organism must work harder during development to counteract adverse nutritional supplies, ultimately optimizing developmental stability over developmental rate. The first semester of thesis work was dedicated to exploring how nutrient supply and developmental rate interact to influence developmental stability in *Drosophila melanogaster* and how the mechanisms driving these interactions may play a role in the phenotypic manifestation of chronic disease in humans.

2.4. Extending Detection to Alzheimer's Disease

After analyzing wing-vein Fluctuating Asymmetry during the first semester, the second semester's work aimed to extend the analysis performed on drosophila wings to drosophila nervous systems. The nervous system is notoriously greedy with the body's energy supply, especially during childhood when crucial brain development takes place. One study estimated that brain metabolism during childhood accounted for 43% of the body's daily energy requirement (Kuzawa et al., 2014). The nervous system is thus especially sensitive to the metabolic environment present during development and adverse environments can lead to impaired brain function. Not surprisingly, conditions such as high blood sugar and diabetes contribute significantly to neurological decline and dysfunction (Rajan et al., 2018). Particularly,

the hormone insulin has been shown to play a tremendous role in the progression of neurogenerative disease such as Alzheimer's disease. It seems that insulin plays an integral role in promoting proper neuronal function as well as managing biomarker modifications. Disruption of this neuronal physiology causes the hallmark cognitive decline observed in Alzheimer's patients and the continuous interplay between insulin-resistance and biomarker progression makes this disease so difficult to treat.

In healthy brains, insulin acts as an important modulator of neurotransmitter release, synaptic plasticity, and membrane polarization, which are important mechanisms for learning and memory formation (Zhao et al., 2015). However, when the brain becomes insulin-resistant, these mechanisms are disrupted as insulin-resistance promotes the dysregulation of biomarkers, specifically tau hyperphosphorylation and beta-amyloid plaque accumulation, causing a breakdown in important cellular processes (Mullin et al., 2017). Conversely, these biomarkers promote further insulin resistance by encouraging microglial inflammatory responses and oxidative stress, additional symptoms of Alzheimer disease (Tonnies & Trushina, 2017). Interestingly, the neurotoxic proteins and agents of oxidative stress observed in Alzheimer's disease are also present in higher concentrations in the peripheral tissues of diabetic individuals versus non-diabetic individuals (Miklossy et al., 2010). Due to shared physiopathology of insulin-resistance in both diseases, it is evident that a common underlying metabolic mechanism contributes to Alzheimer's disease and diabetes mellitus, resulting in many researchers calling Alzheimer's disease "type 3 diabetes" (see figure 2).

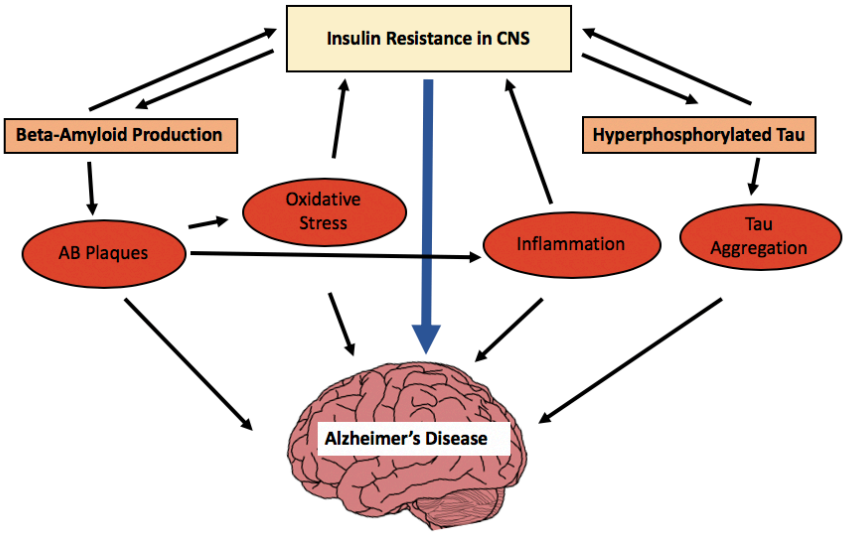

Figure 2. Feedback loops implicated in Alzheimer's disease biomarkers and insulin resistance. As biomarker development progresses, further insulin resistance is promoted in brain tissue - one of the many reasons Alzheimer's disease is so difficult to manage and treat.

It is likely that the mechanisms that drive these commonalities between both disease were programmed during early-life experiences and therefore similar early screening methods could be applied to both diseases. The second semester project worked to further demonstrate this metabolic similarity between diabetes and Alzheimer's disease by expanding the first semester's

work on diabetes to include models of neuronal insulin resistance using *Drosophila melanogaster* nervous systems. By demonstrating that the same metabolic stress capable of inducing diabetes mellitus in drosophila is also capable of influencing nervous system symmetry in drosophila, early screening methods used for diabetes mellitus could also possibly be used for early detection of Alzheimer's disease.

Several studies have already solidified various phenotypic asymmetries as biomarkers of Alzheimer's disease, such as atrophic asymmetry in peripheral Alzheimer's patients' nervous systems (Derflinger et al., 2011), although these detection techniques are expensive and require substantial disease progression. By establishing a shared manifestation of metabolic disorder in both wing asymmetry and nervous system asymmetry of drosophila, the research could support applying the fingerprint analysis used in diabetes detection as suggested by Morris et al (2016) to Alzheimer's disease as well. Early detection for Alzheimer's disease could therefore be greatly enhanced as it could rely on more accessible phenotypic markers.

The current project in its entirety worked to conclude that 1) fluctuating bilateral asymmetry is a viable biomarker of both diabetes mellitus and Alzheimer's disease and 2) a shared metabolic deficit is the driving force behind both diseases in order to facilitate the development of low-cost, early screening methods.

3. Methods

3.1. Fly Rearing

Wild-type Canton-S stock flies were obtained from Bloomington Drosophila Stock Center. Stock cultures were maintained on Carolina Blue instant media (Carolina Biological Supply Company, Burlington NC) under controlled conditions until introduced to control (low sugar, 0.15 M sucrose) or treatment media (medium sugar, 0.5 M sucrose; high sugar, 1.0 M sucrose; and very high sugar, 1.5 M sucrose) for experimentation. Recipes for all control and treatment media were adapted from *Musselman et al.(2018)* and can be found in table 1. The recipe for 1.0 M adapted from *Musselman et al. (2018)* has been well-established as a recipe that induces diabetes in drosophila. Adults stock flies were anesthetized with CO_2 and randomly introduced to either a control medium or one of three treatment media. Adult flies were left to lay eggs on the new media for 48 hours before removal. Control and treatment vials, now containing eggs, were kept in an Thermo Fischer Model 3900 Series incubator at 26 degrees Celsius, 50-60% humidity, and on a 12-hour photoperiod. Developmental rate was accessed using larval instar stages as indicators of developmental progression (see figure 3).

Table 1. Recipes for drosophila control and three treatment media. A) control media with 0.15 M sucrose, B) treatment #1 media with 0.5 M sucrose, C) treatment #2 media with 1.0 M sucrose, D) treatment #3 media with 1.5 M sucrose.

A

0.15 M Sucrose	
Agar	5 g
Brewers Yeast	80 g
Yeast Extract	20 g
Peptone	20 g
Sucrose	51 g
1.0 M MgSO4	2 ml
1.0 M CaCl2	3.4 ml
Propionic Acid	6 ml
Distilled Water	add to 1 L

B

0.5 M Sucrose	
Agar	5 g
Brewers Yeast	80 g
Yeast Extract	20 g
Peptone	20 g
Sucrose	171 g
1.0 M MgSO4	2 ml
1.0 M CaCl2	3.4 ml
Propionic Acid	6 ml
Distilled Water	add to 1 L

C

1.0 M Sucrose	
Agar	5 g
Brewers Yeast	80 g
Yeast Extract	20 g
Peptone	20 g
Sucrose	342 g
MgSO4 x 6H2O	2 ml
CaCl2 x 2H2O	3.4 ml
Propionic Acid	6 ml
Distilled Water	add to 1 L

D

1.5 M Sucrose	
Agar	5 g
Brewers Yeast	80 g
Yeast Extract	20 g
Peptone	20 g
Sucrose	513 g
MgSO4 x 6H2O	2 ml
CaCl2 x 2H2O	3.4 ml
Propionic Acid	6 ml
Distilled Water	add to 1 L

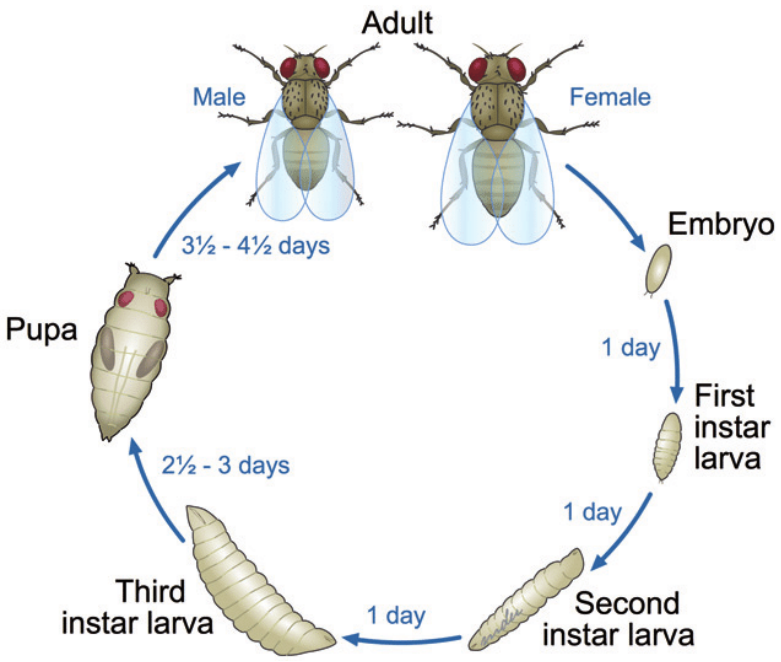

Figure 3. Life cycle of *Drosophila melanogaster*. Adapted from *Ong et al. (2014)*.

3.2. Wing Dissection & Imaging

Adults that completed their life cycles on the control (0.15 M, N = 30) or treatment media (0.5 M, N = 27; 1.0 M, N = 30; 1.5 M, N = 0) were collected for dissection within one week of eclosion. After being anesthetized with FlyNap, both wings were carefully dissected from the body by gently pulling the wing at its base from the thorax. Wings were then mounted onto microscope slides (one wing pair per slide) with Permount® and covered with a coverslip for imaging. Images taken by a 4 megapixel Motic Moticam X3 camera attached to a Nikon Labophot-2 microscope were accessed using Motic Images Plus Software. The lengths of seven specific wing-veins were measured and Fluctuating Asymmetry was calculated as the absolute value of the difference between left- and right-side wing-vein measurements (see Figure 4). These wing-veins were selected due to their relevance in other studies evaluating asymmetry and wing morphology in *Drosophila melanogaster* (Carter et al., 2009). Fluctuating asymmetry scores for the control and treatment groups were compared using an ANOVA single factor analysis or student t-test (significance at $p < 0.05$) to determine if metabolically stressed *D. melanogaster* subjects expressed increased bilateral asymmetry compared to controls.

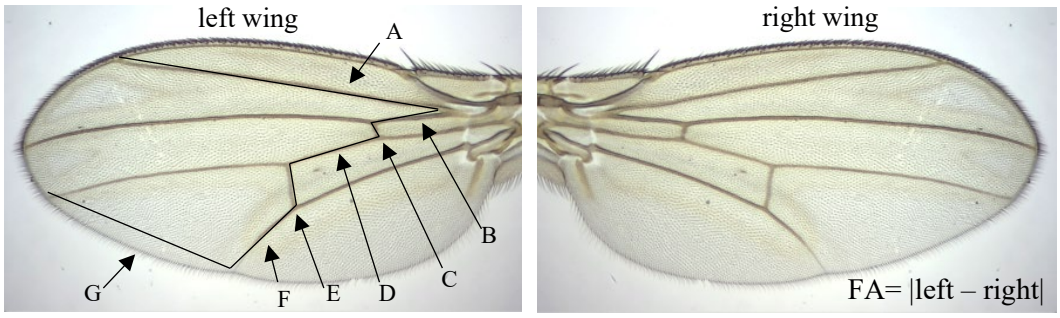

Figure 4. Seven different vein lengths, labeled A-G, were measured on each wing of control and treatment drosophila. (A) longitudinal vein II. (B) longitudinal vein III - only length along the first basal cell. (C) anterior crossvein (D)longitudinal vein IV - only length between intersections with anterior crossvein and posterior crossvein, (E) Posterior crossvein. (F) longitudinal vein V – only length along second posterior cell. (G) distance between distal ends of posterior veins IV and V. Fluctuating Asymmetry was calculated by taking the absolute value of the left vein length minus right vein length for all seven veins.

3.3. Nervous System Dissection & Immunofluorescence Staining

Unfortunately, nervous system immunofluorescence staining and analysis could not be completed this semester, due to suspension of student research in response to COVID-19. Nonetheless, the protocol for immunofluorescence staining was adapted from Manning and Doe (2017), and modified for work at Longwood University. Third-instar larvae will be collected, bleached, and fixed twice in preparation for immunofluorescent antibody staining of nervous systems. Once fixed, larvae will be incubated with Rat-Elav-7E8A10 primary antibody (University of Iowa Developmental Studies Hybridoma Bank, Iowa City, IA) for five days followed by incubation with dye-labeled secondary antibody for 8 hours. After being successfully stained, larval nervous systems will be mounted on microscope slides and visualized using a Nikon Labophot-2 microscope with fluorescence attachment. Images taken with the fluorescent microscopy will be used to assess the degree of asymmetry present in larval nervous systems subjected to the different metabolic conditions. The images taken during fluorescent microscopy will be subjected to morphological measurements of nerve branching points measurements using ImageJ software, to compare left-side and right-side characteristics of each larval nervous system. These data will be collected for both treatment and control groups, and compared using an ANOVA single factor analysis or student t-test to determine if metabolically stressed *D. melanogaster* subjects express increased bilateral asymmetry compared to controls.

4. Results

4.1 Developmental Rate

Notable differences in developmental rate were observed between the 0.15 M, 0.5 M, and 1.0 M populations (figure 5). The 1.5 M sucrose medium did not produce any adult drosophila and only three first-instar larvae were noted during the entirely of the observation period. The drosophila raised on 0.15 M sucrose experienced the most rapid growth, followed by the 0.5 M drosophila, which typically reached life-cycle stages one to two days after the 0.15 M drosophila. Drosophila raised on 1.0 M sucrose showed noticeable delay in growth, reaching life-cycle stages up to five days later than 0.15 M flies. Differences in population size among the aforementioned three groups were also observed, although not quantified, as demonstrated in figure 6.

4.2 Wing-Vein Analysis

The data showed no significant difference in Fluctuating Asymmetry for any of the seven wing pairs between the 0.15 M (N=30), 0.5 M (N=27), and 1.0 M (N=30) drosophila populations (figure 7). The 1.5 M (N=0) sucrose medium did not produce any adult drosophila and thus was not included in any statistical analyses. Variations in the p-values comparing wing-vein pairs across the two groups did, however, indicate that some wing pairs may be more susceptible to Fluctuating Asymmetry than others. Comparison of wing-vein A (see figure 4) resulted in a p-value of 0.13 while wing-vein G yielded a p-value as high as 0.96, demonstrating a notable difference in the predictive value for each wing-vein.

Additionally, comparing asymmetries across the three groups by sex did result in significant differences for certain vein pairs (figures 8 & 9). Females of the 1.0 M population showed a significantly higher degree of Fluctuating Asymmetry for wing-vein D than the 0.15 M population with a p-value of 0.02, whereas comparing the same wing-vein between the males of the two population resulted in a p-value of 0.97, indicating a large difference between the two sexes. Additionally, comparing Fluctuating Asymmetry in wing-vein A of the 0.15 M females indicated that they were more asymmetric ($p = 0.006$) for this wing-vein than the 0.5 M females. Wing-vein B also proved to significantly more asymmetric ($p = 0.03$) for 0.5 M males compared to 1.0 M males. Even within the same population, females and males displayed differences in Fluctuating Asymmetry (figures 10, 11, & 12). For the 1.0 M population, comparing Fluctuating Asymmetry in wing-veins B and E between males and females resulted in p-values as low as 0.02 and 0.08, respectively (figure 12). Wing-vein E also proved to be significantly different between the males and females of the 0.5 M populations ($p=0.02$).

4.3 Nervous System Analysis

Unfortunately due to the suspension of student research at Longwood University in response to COVID-19, nervous system immunofluorescence staining and analysis could not be completed. Staining and analysis will be continued in the near future.

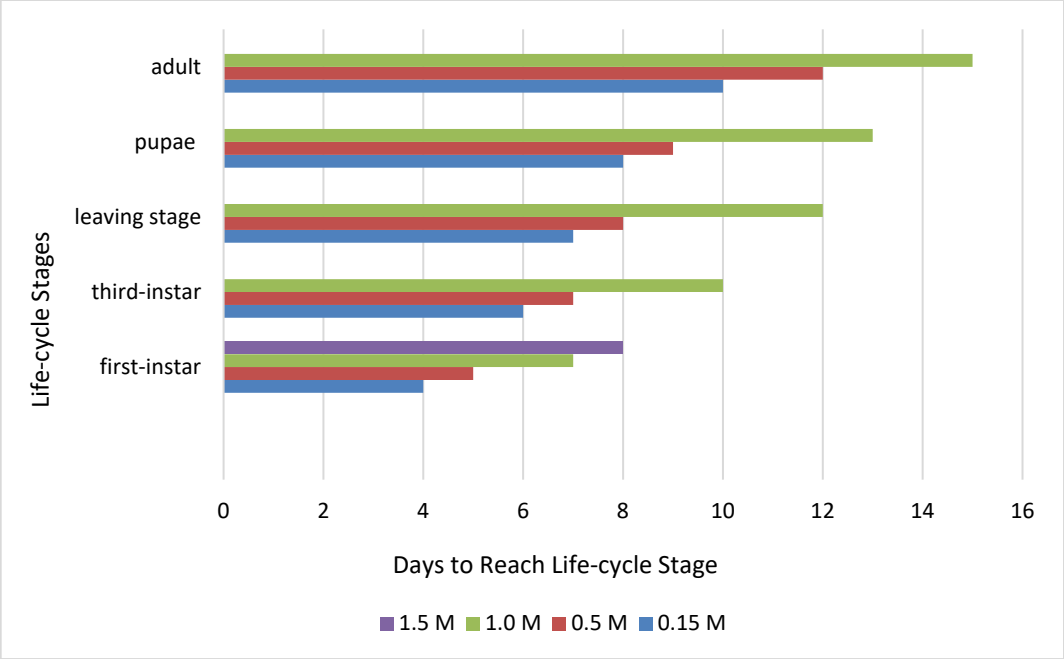

Figure 5. Developmental rate assessed by the number of days taken to reach each life cycle stage by population. Since each individual vial could not be monitored multiple times each day, one representative vial from each population was placed in front of a camera in order to monitor developmental rates across the four populations (hence, no error bars).

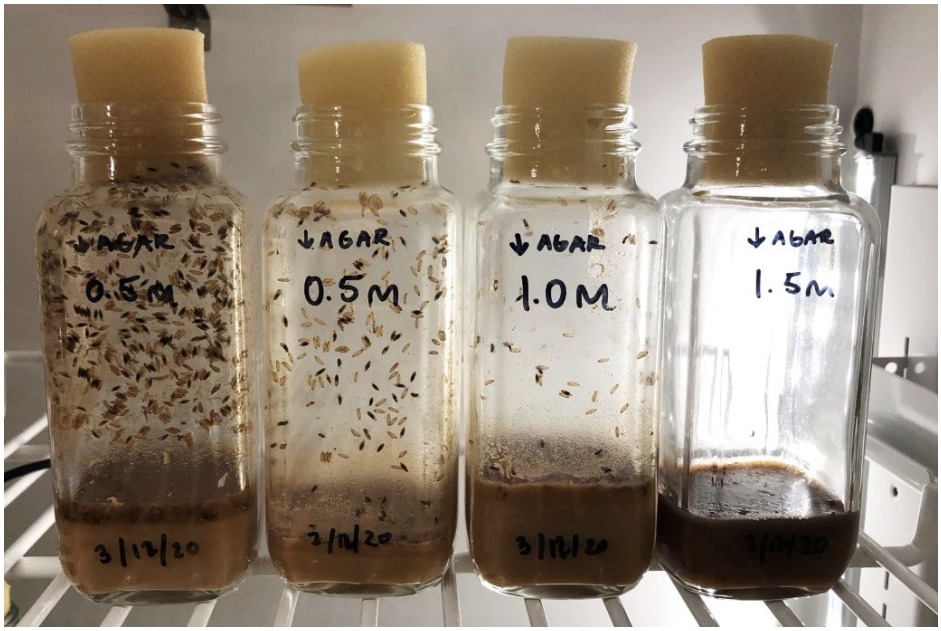

Figure 6. Population growth of 0.15 M, 0.5 M, 1.0 M, and 1.5 M populations twenty days after eggs were laid on each medium. A clear gradient in population size can be observed across all four groups. The 1.5 M sucrose medium did not produce any adult drosophila and thus was excluded from statistical analysis.

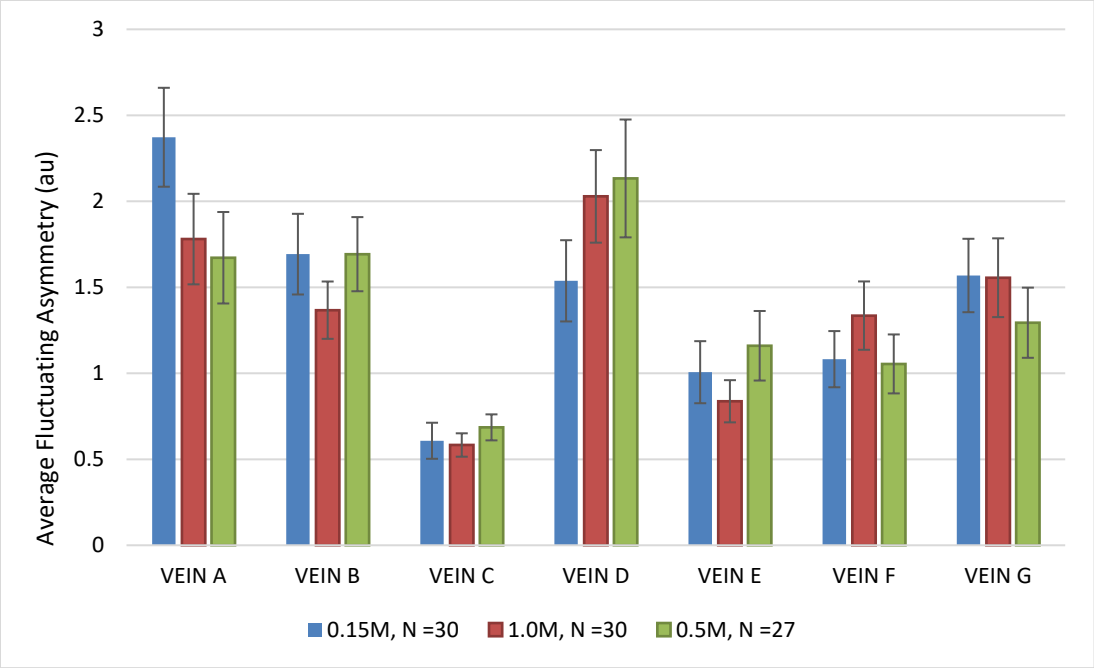

Figure 7. Comparison of average Fluctuating Asymmetry by wing-vein observed in 0.15 M, 0.5 M, and 1.0 M populations. Bars indicate standard error.

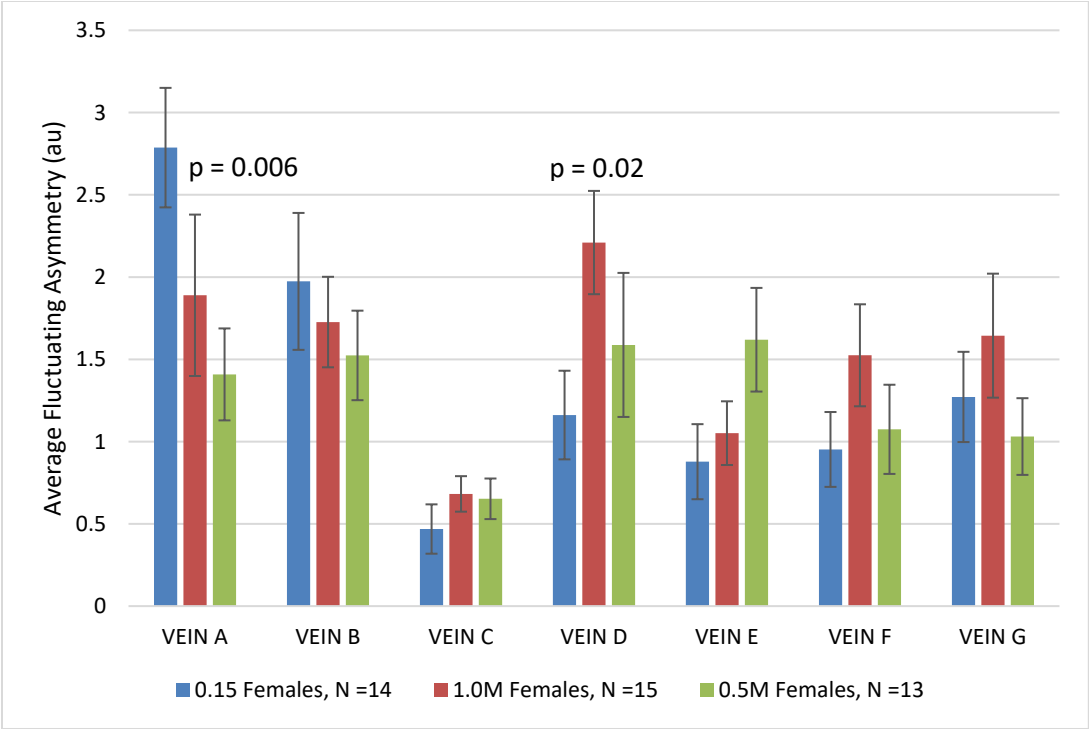

Figure 8. Comparison of average Fluctuating Asymmetry by wing-vein observed in **females** of 0.15 M, 0.5 M, and 1.0 M populations. Significant observed between 0.15 M females and 0.5 M females for wing-vein A ($p = 0.006$) as well as 0.15 M females and 1.0 M females for wing-vein D ($p = 0.02$). Bars indicate standard error.

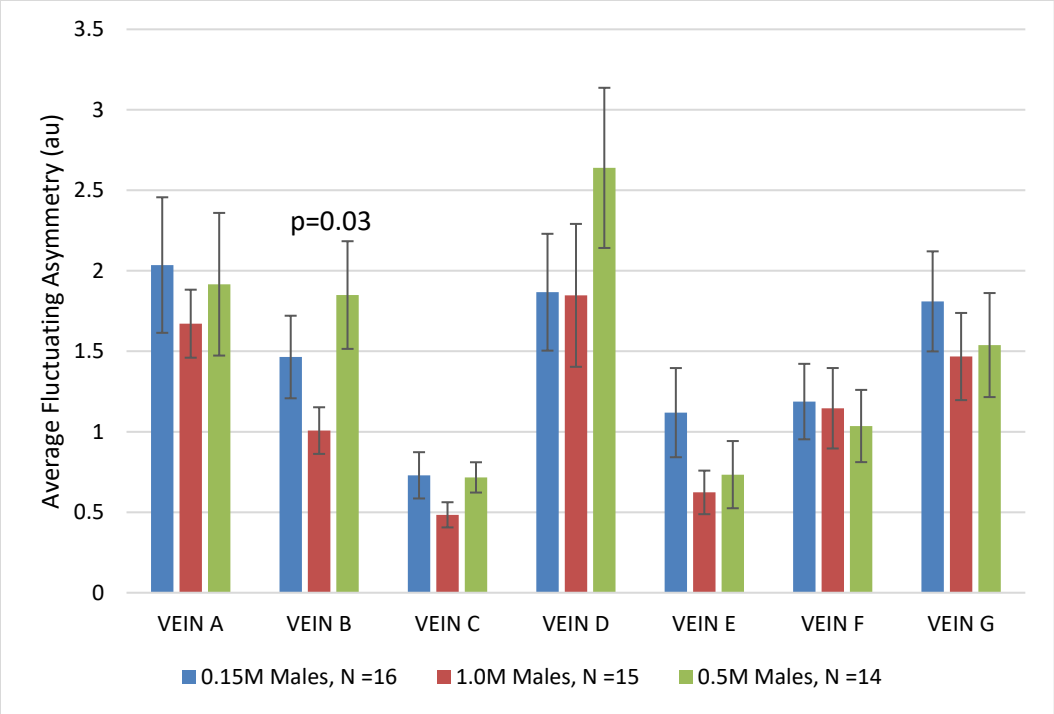

Figure 9. Comparison of average Fluctuating Asymmetry by wing-vein observed in **males** of 0.15 M, 0.5 M, and 1.0 M populations. Significance observed between 0.5 M males and 10. M males for wing-vein B (p=0.03). Bars indicate standard error.

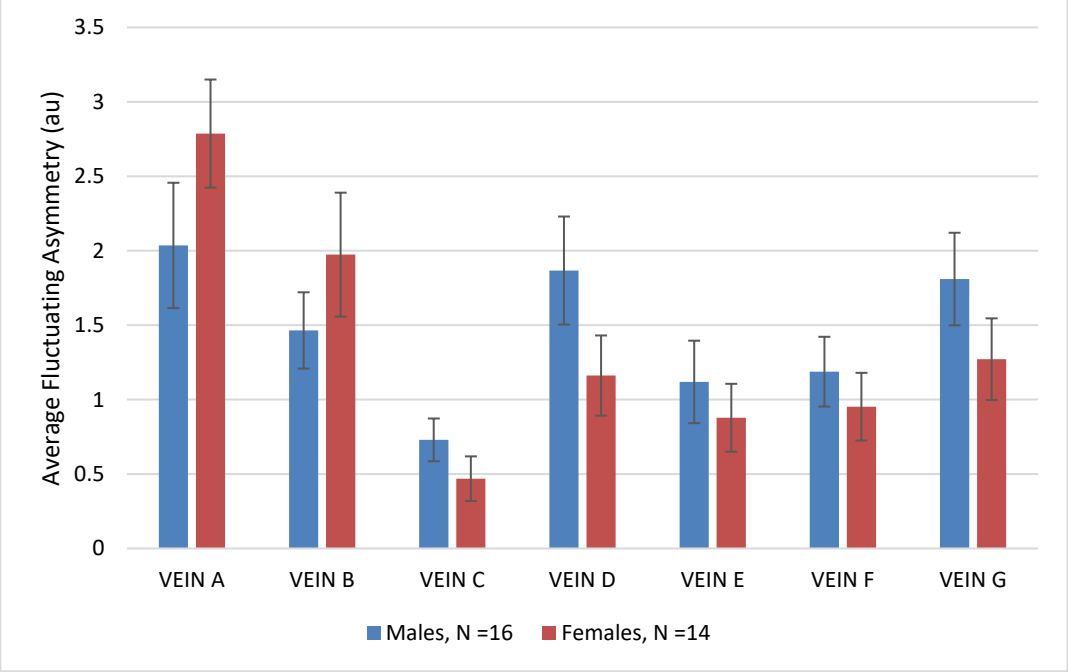

Figure 10. Comparison of average Fluctuating Asymmetry by wing-vein between males and females of 0.15 M population. Bars indicate standard error.

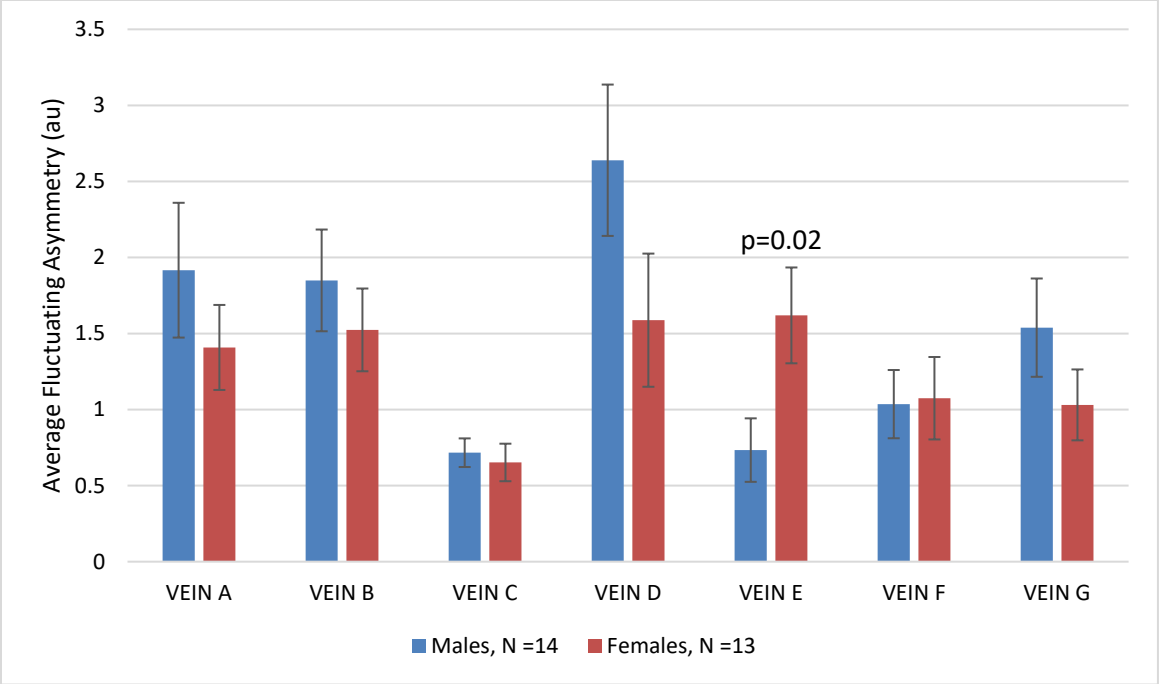

Figure 11. Comparison of average Fluctuating Asymmetry by wing-vein between males and females of 0.5 M population. Significance observed between 0.5 M males and females for wing-vein E ($p = 0.02$). Bars indicate standard error.

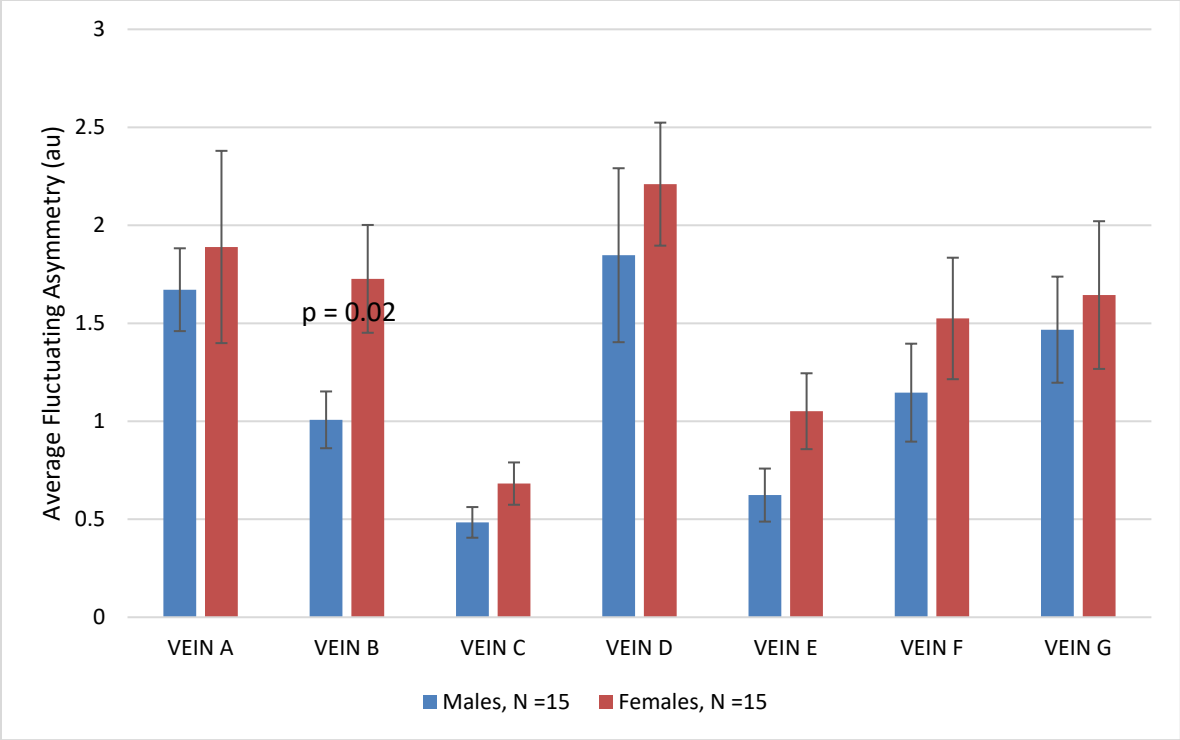

Figure 12. Comparison of average Fluctuating Asymmetry by wing-vein between males and females of 1.0 M population. Significance observed between 1.0 M males and females for wing-vein B ($p = 0.02$). Bars indicate standard error.

5. Discussion

5.1. *Data Interpretation and Future Directions*

As this project continues to develop, the data collected during these initial experiments has provided valuable insights into the interactions between developmental rate, metabolic environment, and Fluctuating Asymmetry and their combined role in establishing developmental stability. Clear differences in developmental rate and resulting population size were observed between the 0.15 M, 0.5 M, and 1.0 M groups. Although the data did not produce significant trends in Fluctuating Asymmetry on a population level between the three groups, significant differences in Fluctuating Asymmetry could be observed between the males and females of the same population as well as between females or males from different populations. It is worth noting that differences in wing size between males and females may account for some of these observations and that vein lengths will be normalized by average length of vein A for the respective sex in future analyses. Nonetheless, these findings suggest that metabolic stress may in fact produce differences in development to some degree. The question remains as to whether slower developmental rate in response to metabolic stress may work to mitigate Fluctuating Asymmetry or if ideal metabolic conditions optimize faster growth rates over symmetry. In other words, a high sugar diet may produce a more asymmetrical population if developmental rate was not able to alleviate the burden of metabolic stress. Conversely, ideal metabolic conditions may produce a more symmetrical population if developmental rate is slowed. Future experiments could equilibrate the developmental rate (i.e. through temperature adjustments) of the two populations so as to observe differences in Fluctuating Asymmetries between “high sugar” and “low sugar” drosophila that are forced to develop at same rate.

Furthermore, future studies could involve comparing genetic models of diabetes or Alzheimer’s disease (i.e. using drosophila with predetermined genetic markers for disease) with drosophila raised on our modified mediums to observe how Fluctuating Asymmetry may differ across the various groups. Because our flies may be optimizing two different developmental strategies in response to their metabolic environments, using our 0.15 M medium as a “control” may be inaccurate. Thus, using Fluctuating Asymmetry observed in established models of disease as controls and comparing our drosophila to this baseline instead may produce more conclusive results.

In addition to producing the aforementioned trends, this project has also established the necessary procedures and technologies to perform nervous system immunofluorescent antibody staining during future experiments. Perhaps wing-vein symmetry may not be the most adequate phenotypic marker of developmental asymmetry due to selective pressure to maintain wing-vein dimensions required for proper locomotion (Ray et al., 2016). Future projects must work to identify drosophila traits that are more susceptible to Fluctuating Asymmetry during development, and thus reflect developmental instability more prominently for disease detection purposes. For example, human fingerprints do not provide a necessary adaptation for survival or reproduction so their symmetry may be less conserved when undergoing development, as this trait is less essential. On the contrary, drosophila wings are paramount to their survival and reproduction so perhaps wing-vein symmetry is more highly conserved during development. A less essential drosophila trait like thoracic bristle symmetry may be subjected to less selective pressure during development as symmetry of thoracic bristles is not vital for survival or reproduction, possibly resulting in a higher susceptibility to Fluctuating Asymmetry compared to

drosophila wings (Petavy et al., 2006). Therefore, extending the analysis to different phenotypic traits – particularly nervous systems, which are especially metabolically sensitive and whose morphology can vary widely without direct functional consequences – may yield more significant results.

5.2 Extending Trends to Disease Detection

Developing early detection technologies using asymmetry requires a deeper understanding of the processes that drive developmental stability, including but not limited to developmental stability and metabolic environment. Three major trends observed during this project, while not entirely significant, have provided valuable insights into the interplay of developmental influences and opened new doors for further research. Firstly, the clear differences in developmental rate support the hypothesis that organisms in ideal environments may favor faster growth by sacrificing developmental stability since the environment is likely to offer less stressors compared to a less idea environment (Morris et al., 2012). The organisms raised in a high sugar environment likely had to "work harder" during development to maintain strong developmental stability so as to survive in a less than ideal environment during adulthood, thus resulting in a slower developmental rate. While developmental rate is not as straight forward in humans as it is in drosophila, this observation suggests that the timing of developmental stages is important in producing a symmetrical organism and that external signals may affect rate of development, not just act directly on morphology. Further research on how developmental rate may mitigate the effects of environmental stress is crucial in understanding an individual's developmental stability and thus is greatly needed to support early screening technologies.

Secondly, the variability in predictive potential of wing-veins suggest that some phenotypic aspects of the body may be either more conserved during development or more susceptible to deviations resulting from stress during development. Morris et al. (2016) observed a similar trend in their fingerprint study as only three fingerprint pairs showed significant ability to predict type 2 diabetes mellitus. With this information in mind, future studies can include the analysis of different wing-veins or vein patterns as well as novel morphological markers in order to identify which phenotypic characteristics may be more (or less) susceptible to Fluctuating Asymmetry under varying metabolic conditions. Such research could support extending the search for additional phenotypic "disease patterns" to humans as well in order to development more precise disease assessment protocols.

Thirdly, the observation that females tended to have higher rates of Fluctuating Asymmetry, particularly for the 1.0 M population, indicates that body size may also be a contributing factor to developmental stability. Female drosophila are noticeably larger than their male counterparts, yet exhibit the same developmental rate as males. The need to produce a larger organism during the same timespan could certainly provide additional developmental stress during female development. For human disease detection, perhaps additionally information such as birth weight could be used in conjunction with measured asymmetries in assessing an individual's risk for developing metabolic disorders later in life. In fact, babies born with macrosomia (significantly higher birth weight than average) are at increased risk for metabolic disorders during childhood, and consequently throughout the rest of their lives (Mayo Clinic, 2018). Incorporating the role of body size in developmental stability could serve as an important consideration when assessing disease risk of an individual later in life. It has also been established that female drosophila exhibit different nutrient demands, energy allocation, and insulin signaling than male drosophila.

While sex is a more complicated case for humans, this observation supports that screening techniques may need to be calibrated depending on sex for optimal results. While some phenotypic manifestations of disease may provide high predictive values for one sex, those manifestations may prove insufficient for the other sex. More research in determining sex-dependent differences in the developmental origins of disease is needed.

5.3 Conclusions

Designing technologies based on measurements of developmental stability to predict an individual's risk of developing chronic diseases, particularly diabetes mellitus, could transform preventative medicine. Fluctuating asymmetry is an assessible, measurable phenotypic manifestation for developmental stability and thus could prove as a key factor in such disease detection technologies. This project aimed to elucidate metabolic stress as a driver of fluctuating asymmetry as well as the role of developmental rate in either mitigating or exacerbating the observed asymmetries. Experimentation resulted in notable differences in developmental rate between the 0.15 M, 0.5 M, 1.0 M and 1.5 M populations and, although wing-vein analysis results were thus far inconclusive, this project in totality has illuminated the role of developmental rate, metabolic environment, and fluctuating asymmetry in establishing developmental stability. This project has also identified other factors - such as selective pressures of certain bilateral traits, body size, and sex - that could be incorporated into early screening protocols to make predictions more accurate for each individual. Further experimentation is needed to establish significant trends between developmental rate, metabolic environment, and fluctuating asymmetry in order to support the development of early screening methods for diabetes mellitus and other metabolic disorders. As rates of chronic disease continue to rise, the need for more consistent early screening methods will only increase and thus continued research in this field is greatly needed.

6. Acknowledgments

I thank Dr. Bjoern Ludwar for his invaluable mentorship and guidance throughout this project. I also thank my examining committee of Dr. Benjamin Campbell, Dr. Ruth Holliday, and Dr. Brandon Jackson for the constructive criticism and feedback on this work. Lastly, I thank the committee of Senior Honor's Research for the opportunity to conduct and present this research.

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