THE GENETIC AND PHYSIOLOGICAL BASIS OF TOTAL ENERGY BUDGET IN DIFFERENT NUTRITIONAL ENVIRONMENTS

A Dissertation
Presented to
the Faculty of the Graduate School
at the University of Missouri

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

by
ANNA PERINCHERY-HERMAN
Dr. Elizabeth King, Dissertation Supervisor
MAY 2020

The undersigned, appointed by the Associate Vice Chancellor of the Office of Research and Graduate Studies, have examined the dissertation entitled

THE GENETIC AND PHYSIOLOGICAL BASIS OF TOTAL ENERGY BUDGET IN DIFFERENT NUTRITIONAL ENVIRONMENTS

presented by Anna Perinchery-Herman, a candidate for the degree of doctor of philosophy, and hereby certify that, in their opinion, it is worthy of acceptance.

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Professor Elizabeth G King

_____

Professor Paula McSteen

_____

Professor James Birchler

_____

Professor Gregory Blomquist

For my parents, Vinny and Princy, who pushed me
For my sisters, Christine and Caitlin, who believed in me
For my love, Stephen, who never let me give up.
Thank you.

ACKNOWLEDGMENTS

There are several people, without whom, this dissertation would not have been impossible. First, I would like to thank my advisor, Dr. Elizabeth (Libby) King for the opportunity to work in her lab. This research would have been impossible without the resources and mentoring provided in lab. A special thanks to my lab mates, current and former including: Dr. Enoch Ng'Oma, Mathangi Ganesan, Zeke Elkins, Paul Petrowski, Elizabeth Jones, Patricka Williams-Simon, Elizabeth LoPresti and Patrick Stanley and especially the undergrads that assisted me on this project: Stevie Winingear, Hanna Kleiboeker, Dennis Hernandez, Ethan Redding.

Additionally, I would like thank several people in the Division of Biological Science, and my dissertation committee: Dr. Jim Birchler Dr. Paula McSteen, and Dr. Greg Blomquist. Their suggestions and guidance were very helpful. I would also to extend my thanks to the following from both the Biology department and the Graduate School: Dr. Miriam Golomb, Dr. Laura Page, Dr. Michael Garcia, Dr. Bethany Stone and Dr. Aditi Mishra for their advice, support and encouragement.

Lastly, I cannot thank my family and friends enough. There was a village cheering me on as I went through graduate school. I would like to thank the following family and friends for the endless love and support: Sicy Davis (Pink Ammaie), Tessy and Sunny Perinchery (aka Vellamma and Vellappan. Vellamma, I wish you could have been here to see it) SO. MANY. COUSINS. My in-laws: David, Christina and Tom, John and Margie. As well as: Dante's Inferno Book Club (Amy, Mary, David, Fr Reg, & Fr. Pachomius and Fr. Rich); friends from Newman University: Kat (nee Mercer) Barker and Angie Ayers, Dr. Casey McNeil & Dr. Alan Oberley; and instructors from Blue

Valley High School: Ms. Asquith, Ms. Taghizedeh, Ms. Toneva, Ms Sieve, and many others.

And lastly, there are people in my life that I ABSOLUTELY could not have done this without: their support, encouragement, love, patience and humor have been what kept me writing on days with writer's block, persisting in lab when experiments didn't work and continuing to code when all I wanted to do was give up. Mom and dad (Princy and Vinny) were the first people to encourage my interest in science from kitchen experiments to the countless visits to science museums. Their endless support has been priceless. I have the best siblings in the world: Caitlin, Christine and Paydon, thank you for your humor and love. I couldn't have finished without my two partners in crime, my sisters, Christine and Caitlin: your listening ears, shoulders to cry on and fierce (absolutely fierce) encouragement kept me from ever giving up. A special shout out to Bella (the sweetest dog in the world), who kept me smiling (and my feet warm) these last few months and reminding me there's nothing a walk and a snack can't fix. Finally, my husband, Stephen. We met in grad school and now we're standing at the finish line together. Thank you for encouraging me when I didn't know how to move forward. Thank you for your unshaking faith and optimism. Your endless support, encouragement, and absolute refusal that I would quit. Look at us. We made it. You are the best thing that has ever happened to me, and I couldn't have done this without you.

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Abstract

Organisms need to adapt to dynamic environments over time. An organism consumes and stores a finite amount of resources that are used for all daily tasks. In order to survive and thrive, they must allocate these finite resources to different life history traits like reproduction or somatic growth. In order to understand this process, I examined the genetic and phenotypic variation in macromolecule content, estimated heritability for these phenotypes, and studied the effects of selection on macromolecule content.

In my first study, I used the genetic mapping population, the *Drosophila* Synthetic Population Resource (DSPR), to measure macromolecule content and mapped the genetic loci responsible for carbohydrate, lipid and protein storage on different diets in *Drosophila melanogaster*. I measured the effect of nutritional environment on overall fly composition. By using the energy budget assays, I showed that there is phenotypic variation in response to diet, the genotypes responsible for nutrient content storage are plastic and that there are multiple genomic loci of interest.

Nutrient acquisition increased according to diet composition, with DR having the lowest amount and HS having the highest. The exception to this pattern was glycogen. On the C diet, lipid and carbohydrate amounts correlated together. Overall, protein consistently correlated with all other macromolecules between 0.2 and 0.3 correlation. In my second study, I estimated the heritability of lipid, carbohydrate, glycogen, and protein contents across three different diets using a half-sibling design experiment, using flies from a genetically diverse outbred population generated from the DSPR. I showed differing heritability for different macromolecule contents across nutritional

environments. This suggesting not only does nutrient content change based on the particular environment a genotype is in, but that these phenotypes are heritable.

In my final study, I tested the effects of female fruit flies undergoing selection for 30 generations. I measured protein, lipid, soluble carbohydrates, and glycogen amount in ovaries and somatic tissue across three different diets across three different selection regimes and found that selection treatments did not significantly impacted macromolecule content. However, diet did. Strikingly, for carbohydrates specifically, patterns of acquisition remained the same in both the base population and after thirty generations of selection regardless of selection regimen.

Unlike previous studies, I focused on the impact of diet and measured all four energy budget components on the same individual flies. This allows a wider understanding of resource allocation in different environments. I found that there was variation in macromolecule content acquisition. It is a heritable phenotype, and that diet was more influential in macromolecule content allocation than selection treatment.

Chapter 1: Introduction

All organisms acquire energy to store and for daily use. These nutrients can be allocated in a number of different ways: to storage, to lifespan, and to reproduction, and this can vary between individuals within a species (van Noordwijk and de Jong 1986; Reznick *et al.* 2000). Organisms must work within this finite budget (Begon *et al.* 1996). The energy budget consists of all acquired resources that can be used for work and have been assimilated or stored. The amount acquired varies organism to organism (Simmons and Bradley 1997; Jervis *et al.* 2008) A portion of all consumed energy is lost as heat during metabolic processes(Begon *et al.* 1996; Bharucha 2009). At any given snapshot in time, the total energy budget available to an individual organism consists of the energy stored in three macromolecules: carbohydrates, lipids, and proteins. These are either accumulated via food consumed or biosynthesized for a particular purpose with given nutrients. Organisms vary in how they allocate the energy obtained from food resources (Aguila *et al.* 2013).

Drosophila **as Model Organisms**

In this dissertation, I use *Drosophila melanogaster* as a model organism to study energy budget. Flies are a good model organism for a few reasons. Several things we can use to our advantage: first, they are relatively inexpensive to rear; second, we can generate relatively large population sizes in relatively short amounts of time; third, there is a relatively short period from egg to adulthood. There are several genetic and experimental tools available for use in the fly and we have a well detailed, annotated version of the genome (Zinke *et al.* 2002; Hales *et al.* 2015; Anholt and Mackay 2017).

These are all things that have made *Drosophila melanogaster* a good genetic model organism, Broadly, flies have been used to study many aspects of life: circadian rhythm, developmental biology, neurobiology, cancer, aging, and metabolism (Arking *et al.* 2002; Rulifson *et al.* 2002; Aigaki *et al.* 2002; Lee *et al.* 2008; Hales *et al.* 2015; Musselman and Kühnlein 2018; Mirzoyan *et al.* 2019). Flies are also a good tool to study biological processes present in humans. 75% of the disease-causing genes found in humans have homologs in fruit flies(Ugur *et al.* 2016). Lastly, they are a good tool to study a variety of diseases, including, as emphasized in this dissertation, issues associated with metabolism and nutrition. Scientists in the field have generated flies with insulin resistance, obesity, and hypoglycemia (De Luca *et al.* 2005; Baker and Thummel 2007; Skorupa *et al.* 2008; Musselman *et al.* 2011; Stanley *et al.* 2017).

While there are several advantages to using *Drosophila* there are also limitations to what this amazing genetic model organism can do. It is of course, a tool to studying immensely complex biological processes, and often, it is simpler in that way. Flies are insects whereas humans are mammals. *Drosophila* lay eggs and fly (have wings) and have other behaviors present that are not present in humans and vice-versa. And while humans and flies have some organs in common, there are a number of instances where they have organs with similar functions, but not the same (Bharucha 2009): for example, livers in humans and oenocytes in flies. The liver is a single organ in the human body, whereas oenocyte livers have a *similar function* but are clustered throughout the body, rather than a single organ (Gutierrez *et al.* 2007).

A major focus of study in *Drosophila* is metabolism. Scientists have generated flies with phenotypes similar to cardiovascular disease, and several variations in

metabolism phenotypes (Skorupa *et al.* 2008; Jumbo-Lucioni *et al.* 2010). This dissertation seeks to deepen understanding of the energy budget or total energy reserves in *Drosophila.* Organisms have a finite pool of resources with which to do daily tasks, and knowing what is often stored can provide information on how well an organisms might do in a particular environment or respond to certain nutrient, and if there is a predisposition to other diseases, as well as broader understanding in health and wellness (Padmanabha and Baker 2014). Knowing the composition of total energy reserves also acts as a good measure of diet effectiveness. Hand in hand with energy reserves is body mass (Jumbo-Lucioni *et al.* 2010). Flies have only their bodies to store nutrients. There is variation in body mass across the species, and this can be affected by nutrient environment (Kristensen *et al.* 2011; Moghadam *et al.* 2015; Garlapow *et al.* 2017).

Fly Ecology

There are three major macromolecules that make up the energy budget: lipids, proteins and carbohydrates; carbohydrates can be further broken up into two categories, soluble carbohydrates and insoluble carbohydrates known as glycogen. There are also minor components within the diet, including some minerals and fiber, but as they are minimal and are not used as energy for work and are not a major part of the energy budget and are not found in large quantities within the fly body , we will proceed mainly be dealing with the three main macromolecules listed above. These macromolecules are fundamental parts of development and throughout the lifespan of a fly.

Each of these macromolecules is important to understanding fly biology. Lipid and glycogen are the two major energy sources for a fly. Glycogen is especially

important during starvation and flight (Graves *et al.* 1992; Djawdan *et al.* 1998). Further, increased glycogen is correlated with desiccation resistance (Djawdan *et al.* 1996). Lipid, however, is a higher energy-gram ratio than any other macromolecule making it more efficient for an organism to store nutrients in lipids. Further, lipids are correlated with starvation resistance (Chippindale *et al.* 1997; Djawdan *et al.* 1998; Ballard *et al.* 2008). Lipid and glycogen are especially important in obesity research as glycogen, triglycerol and glycerol are three metabolites that are researched for correlating with obesity (Jumbo-Lucioni *et al.* 2010). Not unexpectedly, increased carbohydrate and lipid storage was the result of selection for starvation resistance (Djawdan *et al.* 1998). Those flies that have extra lipid stores, extra carbohydrate stores, and extra-glycogen stores have a higher resistance to starvation. Fruit fly eggs are mainly lipid and protein. As the zygote develops, the ratio of lipid to protein changes. Just prior to hatching, the egg will be >75% protein and the remaining will largely be lipids with <10% being carbohydrates (Medina and Vallejo 2002). Protein also makes up the muscle in fruit flies. Lastly, the protein-carbohydrate ratio in a fly's diet will affect longevity (Bruce *et al.* 2013).

The Genetics of Complex Traits

Allocation and trade offs

All organisms need to weigh allocating specific resources to key life history traits to survive and thrive. Allocation is the distribution of nutrients for specific tasks. In this dissertation I focus on the allocation of individual macromolecule content: carbohydrate, lipid and protein content. I also consider the content type of nutrient allocation between somatic and reproductive tissue. When more energy is allocated to reproduction, less is available to be directed towards survival. Variation in these traits exists between and

within species (Villamarín *et al.* 2016). Some organisms will allot more nutrients towards reproduction versus somatic tissue; the amounts allocated to different tissues can evolve over time: resources allocated to somatic tissue the first generation the organism is on a particular diet treatment will be different to the amount of resources allocated to somatic tissue several generations later (Clark 1990; Clark *et al.* 1990; Chippindale *et al.* 1993; King and Roff 2010). What is allocated to reproduction cannot be allotted to somatic tissue resulting in a trade-off (van Noordwijk and de Jong 1986; Roff *et al.* 2002a; Arrese and Soulages 2010; King and Roff 2010; Shoval *et al.* 2012; Villamarín *et al.* 2016). This splitting of nutrients between reproductive or somatic tissues is an example of a trade-off. At their core, trade-offs are essentially when an advantageous change for one trait means an unfavorable change for another. These are entirely reliant on the total amount of nutrients available for allocation (Stearns 1989; Zera and Harshman 2001). Based on the nutritional environment, the amount of nutrients stored in the body as lipids, carbohydrates, or proteins will change even as the total amount of nutrients acquired changes. (Wigglesworth 1949; Sgro and Hoffmann 2004**).** The tradeoff between reproductive and somatic tissue in nutrient allocation is a relatively well documented phenomenon (For review see (Sgro and Hoffmann 2004; Flatt 2011). There are a wide variety of storage strategies for different organisms. Sometimes this difference in storage strategy is due to the way an organism reproduces. Take, for example, the case of income versus capital breeder. Capital breeders will reproduce using stored up energy, whereas income breeders will reproduce with energy they are taking in concurrently. Some insects, like moths and butterflies, are a good example of capital breeders, though

fruit flies are more similar to income breeders. Endotherms area good example of income breeders (Stephens *et al.* 2009)

The Genetics of Acquisition and Allocation

Acquisition and allocation often varies among individuals and are influenced by both genetic and environmental factors (Chippindale *et al.* 1998; Reed *et al.* 2010b). Knowing the relative influence of genetic versus environmental factors is vital to understanding how organisms respond to their environment. Calculating heritability allows scientists to predict how a population will respond to selection (Roff 2001). This is best explained by looking at the Breeder's equation **($R=h^2S$)** which describes how a trait can respond to selection. R is the response to selection, h^2 is narrow sense heritability and S is the difference between the parental and population means (Roff 2001). Heritability tells us broadly about the genetics but not the specific loci responsible (Clark 1990; Hoffmann and McKechnie 1991; Brokordt *et al.* 2018).

Like height in humans, the genetics of macromolecule content is complex. We expect multiple genes to be responsible for the content of each macromolecule(Reed *et al.* 2014). This is known as a polygenic trait. Likewise, we expect many genes of small effect rather than a single gene or a single set of a few genes with a rather large effect (Reed *et al.* 2014). For example, Wang et al in their 2005 paper used QTL analysis to identify a potential candidate gene for lipid content. They found a potential candidate gene in miR14. "miR14 suppresses cell death and is required for normal fat metabolism." (Wang *et al.* 2005) This is typical of the literature: scientists will find genes related to macromolecule content and effect it, but not directly responsible for

macromolecule content (Wang *et al.* 2005). To examine the genetics of macromolecule content, we used a mapping population known as the *Drosophila* synthetic population resource (DSPR). The DSPR is a mapping population generated by two different sets of 8 sequenced founder lines that were crossed for 50 generations and inbred for 25 generations to produce recombinant inbred lines.

We used QTL analysis of the DSPR to find regions of interest for macromolecule content in the *Drosophila* genome. Quantitative trait loci (QTL) analysis is when one runs a genome scan and the more a particular phenotype is associated with a specific region in the genome, the higher the peak that indicates a region of interest in the genome(Marriage *et al.* 2014). Once there are potential candidate genes, it is possible to do knock-out—eliminating the expression of a particular gene—or knock-down—to limit but not completely eliminate the expression of a particular gene—screens of these candidate genes to test for a specific effect(Garlapow *et al.* 2017).

Other possible analyses include a genome wide association study (GWAS), a two-line cross QTL mapping and evolve and resequence. GWAS analyses look for single nucleotide polymorphisms (SNPs) (Pitchers *et al.* 2017). The DSPR has great diversity but it is not a wild type population, and certain rare SNPs may not be present in the population altogether. We know, for the DSPR, that there is only one of eight possible haplotypes present at a specific location in the genome(King *et al.* 2012a; b). This makes the DSPR an ideal mapping population for QTL analysis, and less ideal for GWAS. Both require pretty large sample sizes(King *et al.* 2012a; b). The associations found in GWAS are usually pretty weak. Two-line cross QTL mapping is less ideal than QTL analysis because one will get higher mapping resolution with a QTL analysis of the DSPR than a

two-line cross (Lafuente *et al.* 2018). To examine the heritability of macromolecule content, we used a half-sibling experimental design. We mated one male with three different females. The offspring of the three females are half-siblings to each other, and within a single male-female pairing the offspring are full siblings. Half-sibling experimental design set-ups are ideal for estimating narrow sense heritability as we know the genetics of the parents and offspring(Bubliy *et al.* 2000). Lastly, one of the benefits of evolve and resequence is that you have a base population that you run a selection experiment on. You can see some of the major differences in response to selection and you can then resequence to find some of the underlying genetic basis of the trait you are looking for(Turner and Miller 2012). It was outside the scope of this dissertation to resequence our selection flies.

Plasticity

Within an individual, a single genotype in different environments can lead to different phenotypes, a phenomenon called plasticity. Organisms alter their body composition depending on the dietary regime. This is an example of a plastic trait. We see this in many different traits: from time of development to aging; body size to time of reproduction to how and when and where resources are allocated in the body (Blanckenhorn 1998a; Tatar and Yin 2001; Arking *et al.* 2002). It has been theorized that plasticity evolved as an adaption to changes in the environment (Corl *et al.* 2018). Understanding phenotypic plasticity allows us to understand how a single genotype can react in different environments (Fox and Wolf 2006). Plasticity in nutrient allocation is a possible strategy for dealing with different nutrient environments, a situation where organism of many species may find themselves in. Organisms need to adapt to dynamic

environments over time and plasticity is one way organisms can do so. In dynamic environments, those genotypes with the capacity for plastic phenotypes have more strategies available to them to allow for intergenerational change and have the chance for higher likelihoods of thriving. This increases an organism's fitness. To examine the genetics of plasticity, we compared the differences in macromolecule content between diets. This would show how a different genotype has different phenotypes in different nutritional environments.

Conclusion

Organisms respond to their environment; there will be phenotypic evolution of traits in response to a selection. Understanding this energy acquisition is vital because there is only a finite amount of energy for a variety of tasks. The optimal energy allocation can change depending on the amount of nutrients available to an organism. I studied the patterns of nutrient allocation to different macromolecules on different diets, the genetic loci responsible and the plasticity of these traits and how they evolve over time. I studied the tradeoffs that exist in these scenarios and in allocation of nutrients between somatic and reproductive tissue. Additionally, this research allows us to study how organisms adapt constant high nutrient diets and whether or not they will continue to over allocate nutrients to storage while on a constant high nutrient diet. In the past, similar research has been conducted, however, it has not been as comprehensive: macromolecule content has been studied, but less work has been done in about macromolecule content changes in response to diet, and less with the entire energy budget measurements on the same flies. Heritability studies have been conducted, but very few where narrow sense heritability was measured for macromolecule content while

flies were on different diets. The main objectives of this project are: 1) what are the genetic basis of energy budget components in different nutritional environments and 2) how energy budget components evolve in response to different nutrient regimes.

This dissertation is split into three projects. In Chapter 2, I attempted to identify the genetic loci responsible for carbohydrate, lipid, and protein content in adult *Drosophila melanogaster* on different diets. In Chapter 3, I examined the pattern of heredity of carbohydrate, lipid, and protein content in different diets by placing individuals generated from a half sibling population on one of three different nutrient diets. These estimates allow us to determine the proportion of phenotypic variation that is due to variation in genetic factors both within, and in response to, nutrient environments. In Chapter 4, I studied phenotypic and genotypic changes in lipid, carbohydrate, and protein content over evolutionary time in response to selected diet treatment regimes.

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CHAPTER 2: Genetic & Physiological Basis of Energy Budget in DSPR Flies

Abstract

Organisms need to adapt to dynamic environments over time. An organism consumes and stores a finite amount of resources that it uses for all daily tasks. In order to survive and thrive, they must allocate these finite resources to different life history traits like reproduction or somatic growth. We used the genetic mapping population, the *Drosophila* Synthetic Population Resource (DSPR), to map the genetic loci responsible for carbohydrate, lipid and protein storage on different diets in *Drosophila melanogaster*.

We crossed ~250 Recombinant Inbred Lines (RILs) to a standard inbred line. We placed the adult offspring of these crosses onto one of three diets for 10 days: a high sugar, low yeast or control diet. We then used biochemical assays to measure the total energy budget: lipid, carbohydrate and protein content on the same set of flies. Lastly, we performed QTL analysis to identify possible genetic loci responsible for storage of these different components on different diets.

Introduction

Organisms in the wild and the lab alike often alter their body composition (e.g. fat storage) depending on dietary regime (Weindruch *et al.* 1995; Simmons and Bradley 1997; Stelzer 2001). A single individual with a particular genotype living in different environments can express different phenotypes: a phenomenon called plasticity. (Stearns 1989; Fox and Wolf 2006). We see plasticity in many different traits: from time of reproduction to aging (van Noordwijk and de Jong 1986; Tatar and Yin 2001; Arking *et al.* 2002); from body size to time of development (Blanckenhorn 1998b); even how and

when and where resources are allocated in the body (Sisodia and Singh 2012). It has been hypothesized that plasticity evolved as an adaptation to changes in the environment. Plasticity in nutrient allocation is a possible strategy for dealing with variable nutrient environments, a situation organisms of many species encounter (Stearns 1989; Ng'oma *et al.* 2017)

One example of plasticity in different nutrient environments is optimal energy allocation. the y-model hypothesis is when the optimal allocation of energy can change depending on the amount of nutrients available to an organism (e.g. allocation of nutrients between lifespan and fecundity). A finite energy budget affects all organisms; that is, all daily tasks must be completed within an organism's total energy budget. Organisms cannot overextend this budget and it is not infinite (Begon *et al.* 1996; Galgani and Ravussin 2008; Sacristán *et al.* 2017). At any given snapshot in time, the total energy budget available to an individual organism consists of the energy stored in three macromolecules: carbohydrates, lipids and proteins. These three macromolecules, when taken together, represent all the molecules that an organism can use as fuel for work. These are either accumulated via food consumed, or biosynthesized with given nutrients. Organisms vary in how they allocate the energy obtained from food resources(Lee *et al.* 2008; Aguila *et al.* 2013; Mason *et al.* 2016).

Genetic variation in macromolecule content and plasticity is essential as it allows a population to adapt to its environment. Phenotypic plasticity allows an organism to acclimate to its environment. There is also genetic variation within phenotypic plasticity. Organisms require energy in the form of differing amounts of nutrients to fuel various tasks, from reproduction to somatic growth. For example, *D. melanogaster* requires a

bare minimum of proteins and lipids to deposit in eggs (Bownes *et al.* 1988); a fly contains a differing ratio of macromolecules within the body than a fly not focused more on egg number than nutrient deposition in eggs. More than three quarters of the fertilized egg is protein. The remaining is largely lipids and very minorly, less than 10%, carbohydrates (Medina and Vallejo 2002). Nutrient storage can also vary within a species. This genetic variation within a population allows certain individuals to be better suited to their nutritional environment. In order to best understand the genetic basis of these traits, we genetically mapped these traits. Of the many approaches to mapping, we used quantitative trait loci (QTL) mapping using multi parent populations. Other options include: genome wide association studies (GWAS) and two-line cross QTL mapping. GWAS would have allowed us to look at multiple SNPs and any and all SNPs that had even minute implications in energy budget (King *et al.* 2014). Multi-parent populations, however, allow for higher mapping resolution than two-line cross QTL mapping studies (Lafuente *et al.* 2018).

Some members of a species will allocate more resources towards reproduction; therefore, they will require a larger proportion of lipids and proteins to deposit into eggs (Schultzhaus and Carney 2017). Other members will be better adapted to allocating nutrients towards survival and somatic growth. Due to the varying energy requirements of different tasks, the proportion of lipids, proteins and carbohydrates in individuals vary (Muller *et al.* 2017). This splitting of nutrients between differing life history traits and specifically between different macromolecule contents is an example of a tradeoff. At its core, a tradeoff occurs when an advantageous change for one trait means an unfavorable change for another. Based on the nutritional environment, the proportion and amount of

nutrients stored in the body as lipids, carbohydrates or proteins will change as the total amount of nutrients acquired changes (Wigglesworth 1949; Sgro and Hoffmann 2004). A particular genotype that devotes nutrients to protein content on a dietary restriction diet may devote more nutrients to lipid content on a high sugar diet. (van Noordwijk and de Jong 1986; Roff *et al.* 2002b; Arrese and Soulages 2010; King and Roff 2010; Shoval *et al.* 2012; Villamarín *et al.* 2016).

Many of these traits have been studied individually. Reed et al (2010) found that variation in gene by environment (or G by E) interactions accounts for nearly as much variation as genetics alone and more than diet only; they looked at four diets. However, they measured triglycerides and carbohydrates, omitting proteins. Chippindale et al (1996, 1997, 1998) focused on starvation resistance and phenotypic plasticity, but focused on two diets and did not assay for all macromolecules that comprise the total energy budget. Others (Musselman *et al.* 2011), found that alterations in diet composition, rather than calories, control consumption, though this diet manipulation was done in larva . QTL mapping is commonly used, but rarely done in conjunction with diet manipulation (De Luca *et al.* 2005). While there has been previous research on total energy budget—research individually on carbohydrates or lipids contents and their genetic basis—and research in plasticity, few studies detail a combination of both individual energy budget components and plasticity. Considerably fewer search for the genetic basis of either.

To test how differing nutrient environments affected macromolecule content we used flies from a multi-parent mapping population with many Recombinant Inbred Lines (RILs). We fed adult inbred flies one of three different diets: a high sugar, a control, and

a dietary restriction (low yeast) diet, and evaluated the effects of these diets with a modified version of Foray et al's (2012) assay to measure lipids, proteins, soluble carbohydrates and glycogen, an insoluble form of carbohydrates. Because we used inbred lines, we were also able to study the genetic basis of the plasticity of these traits. In this paper, we:

1. measured macromolecule content across 256 lines
2. quantified plasticity in macromolecule content in different nutritional environments
3. attempted to identify the genetic loci responsible for carbohydrate, lipid and protein in different nutritional environments.

Methods

Mapping Population

To study the genetic basis of macromolecule content, variation, and plasticity in these traits, we used the *Drosophila* Synthetic Population Resource (DSPR), a multi-parental mapping population (Flyrils.org; King, Macdonald, and Long 2012; King et al. 2012). The DSPR is a mapping population composed of an A and B population, with a combined total of over 1500 Recombinant Inbred Lines or RILs. It was created by the interbreeding of two separate sets of fully sequenced founder lines, A1-A7 and B1-B7 plus a common founder line AB8, for 50 generations. Because there are only eight haplotypes possible at each genomic position, we can identify QTL peaks by associating the variations in phenotype with variation in the haplotypes throughout the genome (Mackay *et al.* 2012; King *et al.* 2012a; b). Each RIL has been genotyped using Restriction-site Associated (RAD) DNA and founder lines are fully re-sequenced. King et al. (2012 a,b) identified founder genotypes to every RIL using a *Hidden Markov Model*. Full details regarding the generation, statistics and supporting information on the population can be found at King et al (2012 a,b) and at http://FlyRILS.org. Founder line resequencing data can be found at the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (http://www.ncbi.nlm.nih.gov/sra) under accession number SRA051316. RAD DNA data from the RILs can be found under accession number SRA051306 (Stanley *et al.* 2017).

Fly husbandry:

For this experiment, we crossed virgin female flies from 256 B population RILs of the DSPR and with A4 males, an A population founder line (King *et al.* 2012a). The

offspring of this cross are hereby referred to as RIX for RIL cross. This cross was done to mitigate the effects of inbreeding depression while still maintaining a population of genetically identical individuals. The A4 males and RIL females mated for 72 hours and laid eggs on maintenance food to ensure that any experimental differences in the offspring yielded from diet treatments came from food consumption during adulthood. We placed 15 day post-oviposition mated adult females (mated females will lose some stored nutrient content by depositing them in eggs) of the F_1 generation on one of three treatment diets—Control (C), High Sugar (HS), and Dietary Restriction (DR)— for 10 days. These treatments reflect environments of differing nutrient availability: HS reflecting high nutrient availability, and DR reflecting low nutrient availability. The differing nutrient availability was produced by altering amounts of sucrose and yeast in the DR and HS food treatment recipes. The DR diet has half of the yeast the C and HS diet. The HS diet contains nearly seven times the amount of sucrose compared to the C and DR diets (see supplemental, Table S1). Eggs laid by the F1 generation females were not measured in this experiment. After ten days on treatment food, we flash froze the flies in liquid nitrogen and stored them at -80°C until processing. We reared the 256 RIX in six experimental batches consisting of 30 to 100 crosses. Four to six female flies were used for each assay with measurements given per fly. Of 1305 samples, 1200 samples had 6 flies, 2 samples had 5 flies and 103 samples had 4 flies. Some crosses were inadvertently set up more than once. Thus, there were 32 RIX that have biological replicates; biological replicates came from RIXs that were placed in two batches. There were 180 samples where there is only 1 technical replicate (there were a total of 3 plates, where samples run did not have a second technical replicate within the plate). Samples

run at the beginning of the experiment had only one technical replicate before we decided to run samples twice within plate to measure the accuracy of our measurements. Every other sample had at least 2 technical replicates per plate.

Energy budget Assays

We modified Foray et al.'s (2012) assay, a modification of Van Handel's (Van Handel 1985; Van Handel and Day 1988) original assay for mosquitos to measure whole female fruit flies for total amounts of carbohydrates, lipids, and protein content. Briefly, four to six *D. melanogaster* were homogenized in an Eppendorf tube with a stainless steel bead and aqueous lysis buffer solution using a BeadBeater. From this solution we aliquoted 2.5 microliters to measure for proteins. We measured proteins using the Bradford assay and Bovine Serum Albumin as the standard curve. At the end of the proteins assay, we conducted the carbohydrate, glycogen and lipid assays in boro-silicate microplates to ensure no plastic dissolution in the supernatant. We precipitated a pellet from the solution to use to test for glycogen using chloroform-methanol and sodium sulfate. From the coinciding supernatant, we measured carbohydrates and lipids.

For carbohydrates: we evaporated 150 microliters of supernatant to 10 microliters in a borosilicate microplate, added anthrone and heated to assayed for carbohydrates. Following a recommendation from Cheng et al, we departed from Foray et al.'s (2012) assay and increased the length of evaporation time to 40 minutes in a vent hood (Cheng *et al.* 2011). Anthrone reagent was used to measure soluble carbohydrates and glycogen present in the separate pellet in turn. We used a standard curve of dextrose in methanol for both soluble carbohydrates and glycogen.

For lipids, we aliquoted 100 microliters of supernatant and evaporated until dry in a 90-degree water bath. Then, we added sulfuric acid and vanillin to each well. We created a standard curve of oil in chloroform and added it to each well to measure absorbance.

We used standard curves, where the total concentration and macromolecule amount was known in micrograms per microliters, to convert sample absorbance to concentration then to amount. Standard curves were performed on each microplate for each macromolecule measurement. All plates used a within-plate curve, excluding lipids where an overall global standard curve was calculated using values derived from all within- plate lipid standard curves. We calculated a set of global standard curves for each macromolecule for only Plate 1**,** (the first plate run in this experiment). We utilized a global curve for the lipid plates and Plate 1 because the within-plate standard curve did not adequately cover the range of samples within the plate. We measured absorbances per well using a BioTek Synergy H1 Hybrid Reader. Each well was merely a portion of the total solution, requiring the total macromolecule amount per well to be converted to include the total solution, then divided for total amount per fly (Supplement: Conversion Equation). Macromolecule amount is presented as micrograms per fly. Most samples had at least one technical replicate, and we ran them concurrently. We took the mean of these two samples, and moved forward with this value in statistical analysis. We also calculated calorie amounts of each macromolecule according to calorie per gram (protein 3.9 kcal/g; lipid: 8.0 kcal/g, and carbohydrate 1.6 kcal/g) (Phillips *et al.* 1963).

Statistical Analysis

All analysis described below were performed in R (ver. 3.5; R Core Team 2018).

Pre-processing

We attempted to identify any outliers resulting from inaccurate measurements and removed these from the dataset. To do this, we plotted concentrations in a histogram to check for normal distribution of the data, and calculated standard deviations and means for each macromolecule. Outliers were any values three standard deviations away from the mean, or those which varied largely compared to their technical replicate. Any value that met this criteria was removed (see Supplement Figure S1). Extremely high values in the glycogen histogram were due to artifacts while reading the absorbance.

We transformed the collected diet treatment data in order to run certain statistical tests that assume normally distributed residuals. For carbohydrate and protein content on the DR diet, the data was square rooted. We used the natural logarithm transformation for glycogen and left the lipid data untransformed. For the C diet, we took the square root of the data for carbohydrates and lipids to adhere to a normal distribution and used logarithm transformation for glycogen and protein contents. Finally, we used logarithm transformation for HS glycogen data, as we did on the C and DR glycogen data. We did the same for the HS carbohydrates data. We compared the difference in nutrient content between treatments to a normal distribution, but left this data untransformed as it adhered to a mostly normal distribution (See Supplement, Figure S2).

We calculated values for analysis using both absolute values and protein corrected values (proxy for body size). The protein corrected calculations (a body size proxy) did not vary largely from non-protein corrected calculations, nor did they yield significant QTL peaks. As such, we proceeded in our analysis using non corrected data. Protein

corrected plots are included in the Supplementary (Table S1). For the total acquisition plots, we converted non-transformed data from macromolecule amount in micrograms to calories and separated the macromolecules into the three different diets. Within the datasets for the three diets, we summed the 4 macromolecules in order to calculate total acquisition in calories. We then plotted these amounts by RIX on each diet. Lastly, we took the plotted total acquisition by treatment where the RIX where the RIX are denoted by different colors

ANOVA

We performed an Analysis of Variance (ANOVA) to determine the effect of diet treatment on amount of macromolecule, using means for each line. We fit a linear model where the content of a specific macromolecule is modeled by a specific diet treatment. We used the 'lm' function to fit the following linear model:

$$Y_{ij} = \mu + \tau_i + \epsilon_{ij}$$

Where Y_{ij} is the j^{th} observation of response of the i^{th} treatment, μ is the fixed common effect, τ_i is the effect of treatment, and ε_{ij} is the random error present. We then used 'anova' from base R to measure the effect of diet treatment on macromolecule. We followed up with Tukey tests to account for the multiple tests performed.

MANOVA

We performed a Multivariate Analysis of Variance (MANOVA) to determine if there were any significant differences in macromolecule amount between treatments. We used the function 'manova()' from the 'dplyr' package in R and modeled macromolecule by diet treatment (*diplr* package*:* Wickham et al. 2018).

Nutrient Correlation and PCA Plots:

We produced correlations plots for each nutrient comparing pairs of macromolecules then calculated the Pearson's Correlation coefficient. For example, we plotted carbohydrate by glycogen, carbohydrate by protein, and so on. Then, for each pair, we calculated the correlation. We then split up each of these pairwise correlations over diet. For example, within *only* the C diet, we plotted carbohydrate by glycogen, carbohydrate by protein, and so on. Then, we calculated Pearson correlation coefficient for each pairwise correlation. We conducted a Benjamini and Hochberg correction for multiple testing (Benjamini and Hochberg 1995). For the Principal Component Analysis, we took the previously transformed macromolecule data, and converted each component from micrograms into calories. We then ran a PCA with all four macromolecule amounts. We used 'prcomp' to calculate the loadings and plotted PC1 and PC2 (*diplr* package*:* Wickham et al. 2018).

QTL Mapping

The DSPR was derived from eight sequenced founder lines that, after intercrossing and inbreeding to produce RILs, were genotyped. Since there were only eight haplotypes possible at each genomic position, we were able to identify peaks by associating the variations in phenotype with variation in the haplotypes throughout the genome (Mackay *et al.* 2012; King *et al.* 2012a; b).

We mapped by macromolecule for each environment. That is, carbohydrate, glycogen, lipid, and protein measurements were plotted for HS, C and DR. All mapping

was done at once, fitting one model. In addition, we mapped the difference between diets for each macromolecule: DR versus C, DR versus HS and C versus HS.

We used the Haley-Knott Regression (Haley and Knott 1992) to regress our phenotype on the 8 founder haplotype probabilities and then converted the F-statistic to a LOD score. We then ran a permutation test was performed to calculate the significance threshold (Broman and Sen 2009)

We fit the following model:

$$y_i = \sum_{j=1}^{7} p_{ij}\, b_{ij} + e_i$$

Where y_i is the phenotype of the i^{th} RIX, p_{ij} is the probability the i^{th} RIX has the specific j^{th} haplotype at that locus, b_{ij} is the vector of effects for the j^{th} haplotype, and e_i is the vector of residuals. In our dataset, the RIXs we used have a single copy of a founder genotype, and will be heterozygous at a specific genome position given that the original inbred RIL's were crossed with a founder line and homozygous at the same position. We determined a threshold for statistical significance by conducting 1000 permutations of our dataset to calculate the False Discovery Rate. We calculated a LOD score of 10 as a significance threshold. This is higher than previous papers reporting QTL, because each scan was done to account for each diet and then for comparisons between diet. Thus we had to increase the significance threshold to accommodate information from each diet and between diet comparisons. However, as no peaks reached this threshold we used a LOD score of 6.5, the minimum threshold one might get for a *single* (not *multiple* scans as we do here) QTL scan to designates regions of increased interest, while not a significance threshold.

Results

Phenotypic Patterns ANOVA

We measured the nutrient contents of flies within specific diets. For each fly measured, we assayed total soluble carbohydrates, glycogen, lipids, and proteins. We calculated energy content, converted this to calories, and then then summed these nutrients to find total acquisition. The amount of carbohydrates acquired on the DR and C treatments did not significantly differ from one another and were each significantly lower than the carbohydrates acquired on the HS treatment. The amount of glycogen acquired on the DR and HS treatments was the same, and was lower than the glycogen content on the C treatment. Lipids acquired on each diet were significantly different from each other: lowest on the C treatment, highest on HS. Proteins acquired on the DR versus the C diet significantly differed from one other, with a higher amount of protein acquired on the C diet. The amount acquired on HS was not significantly different when compared to the other two diets (See Table 2). This is most unusual in comparison between plots, especially given that the HS diet has the largest amount of nutrients available for storage. There is a great deal of variation in response to diet (See Figure 1). For the total acquisition plots, we can see, as expected, that total acquisition increases, scaling upward as calories increase in the diets. The lowest total acquisition was on the DR diet, highest total acquisition was on the HS diet.

The assay does not distinguish between food within the gut, and food assimilated into the fly body. The food material from the HS diet unassimilated within the gut would have a higher carbohydrate content then the C or DR diet material. Material from the DR diet within the gut would have half the protein than either the DR or HS diets. However,

in comparison to the total fly body, the amounts within the gut are negligible, and they would eventually have been assimilated. So, we will proceed assuming the experimental results are primarily assimilated nutrients. We conducted a separate Analysis of Variance (ANOVA) for each macromolecule: carbohydrate, lipid, protein, and glycogen as well as Tukey tests. We fit a linear model of (transformed) gram per fly of a specific macromolecule against diet. We also conducted Tukey tests, indicating significance in lipid, protein and in carbohydrates.

MANOVA

We measured macromolecule amount per fly on three diet treatments. For each fly measure, we assayed total carbohydrates, lipids, protein and glycogen. Diet treatment caused significant differences in amount for the following macromolecules: carbohydrates, lipid and proteins. Surprisingly, there was no significant effect of treatment for glycogen. This is particularly interesting because glycogen is an insoluble form of carbohydrate. This did not lead to any new conclusions different from the ANOVAs (See Table 3).

Nutrient Correlation Plots and PCA

We produced correlation plots for each nutrient, comparing pairs of macromolecules. Values reported, unless otherwise indicated, are significant. There was little to no correlation between carbohydrates and proteins or carbohydrates and glycogen. The highest correlation value was between lipid and carbohydrate on the C diet (0.2778). It would be curious to see if there are similar mechanisms at work here when there is neither a high sugar content nor low protein content available, and whether or not

lipid and carbohydrate mechanisms or storage values—content values—are more closely linked (Begon *et al.* 1996). Lastly, protein correlations with other macromolecules were most consistent at about 0.2-0.3 correlation (See plots in Supplementary Figure S4*)*. We plotted Principal Component Analysis (See Figure 4) of the total acquisition of nutrients PC1 represents 51.49% of the variance. PC2 represents 26.42% of the variance. Carbohydrate and lipid loaded positively on PC; glycogen and protein loaded negatively. As carbohydrates and lipids increase, glycogen and protein decreases. For PC2, carbohydrates, lipids, and proteins all loaded negatively; glycogen loaded positively.

Quantitative Trait Loci Analysis

We mapped the main effects associated with diet, and also the difference between diets (phenotypic plasticity). A significant association between a region of the genome and the phenotype would result in a QTL peak. The increased resolution, or number of different genotypes assayed, the narrower the QTL peak. A LOD score of 6.5 is the potential minimum threshold value one might get (King *et al.* 2012a; Marriage *et al.* 2014) ignoring the multiple scans we've done here. As such, we used 6.5 as a baseline to indicate regions of increased interest, though not as a significance threshold. No peaks crossed the significance threshold of 10. These quantitative trait loci analysis (QTL) maps show the locations of suggestive regions for nutrient content within different diets as well as between different diets. Overall, the number, location, and maximum peak of each of these QTL differed on the different diet treatments (See Figure 2). We did not see any evidence for QTL shared for any components. However, given that we only identified a few QTL, this does not indicate a lack of shared QTL as it could result from a lack of power. There was one region of interest in the glycogen plot, P1, that reached a

LOD score of at least 6.5, and can be found at the beginning of the 3 chromosome. The lipid, carbohydrate, and protein QTL plots lacked any peaks above a LOD score of 6.5. For the QTL peak that did reach a LOD score of 6.5, we calculated Confidence Intervals as 2 LOD drop intervals (King *et al.* 2012a). An examination of these QTL regions on Flybase did not reveal any obvious candidate genes involved in energy storage processes (See Table 4). However, it is possible future work might identify the relevant functional significance of the causative genes within these region.

Between Diet QTL

On the carbohydrate scan, there was one peak, P2, beyond 6.5 on the 'Between diets' comparison QTL scan in the X chromosome, reaching LOD 6.95 on the C by HS diet comparison. No peaks crested above 6.5 in glycogen or lipid scans. Lastly, for protein, there was one suggestive peak, P3, on the C_HS genome scan on the 2nd chromosome. More interestingly, there was no corresponding peak in HS on the single protein QTL plot (See Figure 3). No peaks reached the significance threshold LOD score of 10. There are multiple reasons for this, mainly: the significance threshold had to increase to accommodate data from these scans, because each scan was done to account for each diet and then for comparisons between diet. Additionally, there was noise present in the assay (see Supplementary: Table S2 for Repeatability estimates).

Table 1. Amounts acquired Means and SE's in micromgrams per fly

	DR	DR	C	C	HS	HS
	Mean	SE	Mean	SE	Mean	SE
Carbohydrate	0.0574	0.003372	0.05776	0.003404	0.05891	0.003386
Lipid	1.0521	0.03240	1.1059	0.03075	1.1137	0.03247
Protein	0.001577	4.629e-5	0.001537	4.743e-5	0.001584	4.883e-5
Glycogen	0.3852	0.02194	0.4281	0.02223	0.3978	0.02591

Table 2. Analysis of variance for macromolecule amount

Source refers to macromolecule, df refers to degrees of freedom, MS means Mean Square, P value indicates significance for carbohydrates, lipid and protein.

Source	**df**	**MS**	**F**	**P**
Carbohydrates	2	660.26	3143.9	2.2e-16
Lipid	2	170.775	967.78	2.2e-16
Protein	2	2913.93	44950	2.2e-16
Glycogen	2	1.15295	1.7986	0.1663
Total	8	3746.118		

Table 3. Multiple analysis of variance for macromolecule amount (MANOVA)

Source refers to macromolecule, df refers to degrees of freedom, MS means Mean Square, P value indicates significance for carbohydrates, lipid and protein.

	df	**MS**	**F**	**P**
Carbohydrates	2	636.86	3030.3	2.2e-16
Lipid	2	147.995	993.83	2.2e-16
Protein	2	2745.88	42780	2.2e-16
Glycogen	2	1.623	2.5727	0.0808

Table 4: Suggestive QTL Peaks

ID	**Macromolecule**	**Diet**	**Peak**	**Confidence Interval**
P1	Glycogen	HS	6.537	2L: 221.5349:221.5420
P2	Carbohydrate	C_HS	6.95	X: 39.97721: 40.47343
P3	Protein	C_HS	6.64	2R: 131.0358: 131.2460

Figure 1: Macromolecule Amounts

The plots below depict the total amount (untransformed, in micrograms) of energy budget components (carbohydrates, glycogen, protein and lipid) on different diet treatments. DR is Dietary Restriction, C is Control, and HS is High Sugar diet. Different line colors indicate RIXs. Mean indicated by black dots and standard error indicated by red bars.

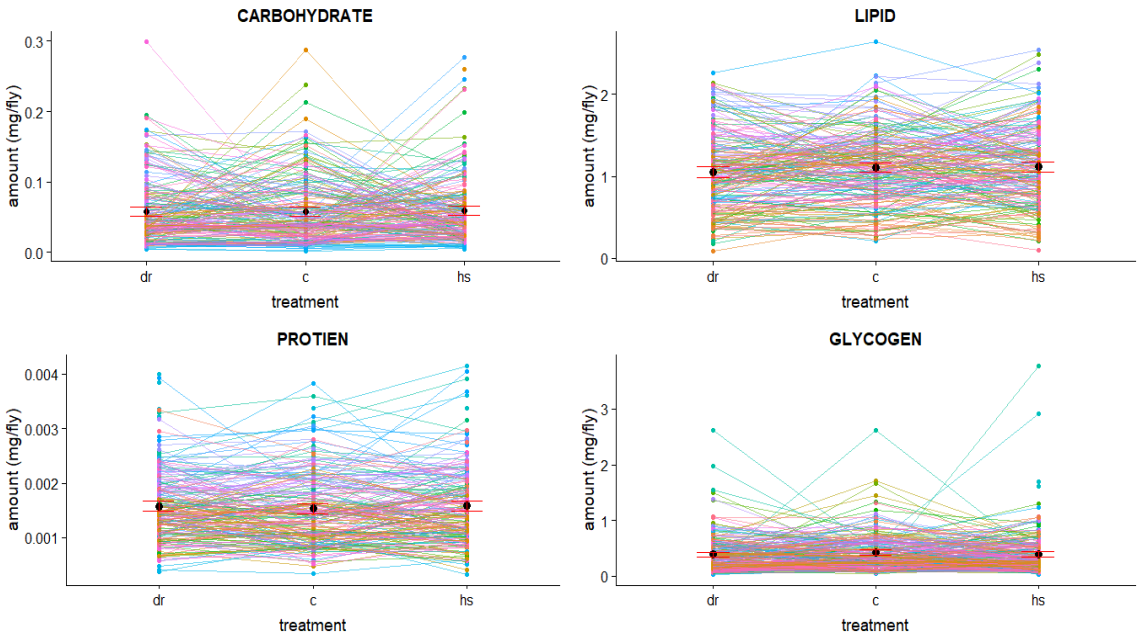


Figure 2: Within Diet Macromolecule QTL Plots

We ran genome scans for components of the energy budget on each treatment. The four plots below depict the Quantitative Trait Loci (QTL) analysis in different diet environments. Chromosome arms are signified by white and gray shaded blocks. Horizontal blue line (LOD= 10) indicates QTL peak significance threshold.

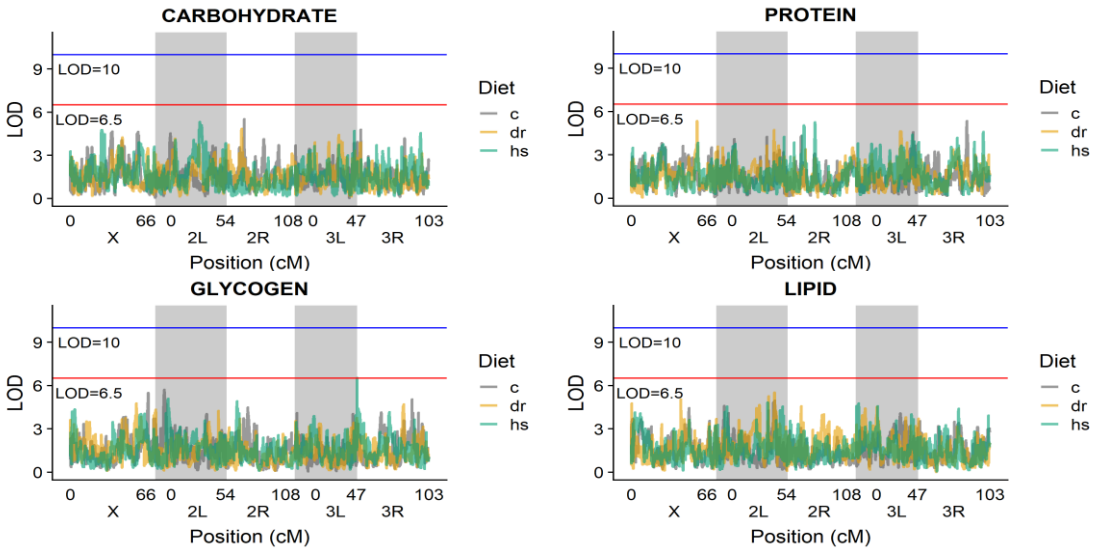


Figure 3: Between Diet Macromolecule QTL Plots

The below four genome scans show the difference between pairs of diet treatments. Blue lines compare the C by DR diet; the red line compares the C by HS diet and the black line shows the comparison between the DR and the HS diet. Chromosome arms are signified by white and gray shaded blocks. Horizontal blue line (LOD= 10) indicates QTL peak significance threshold.

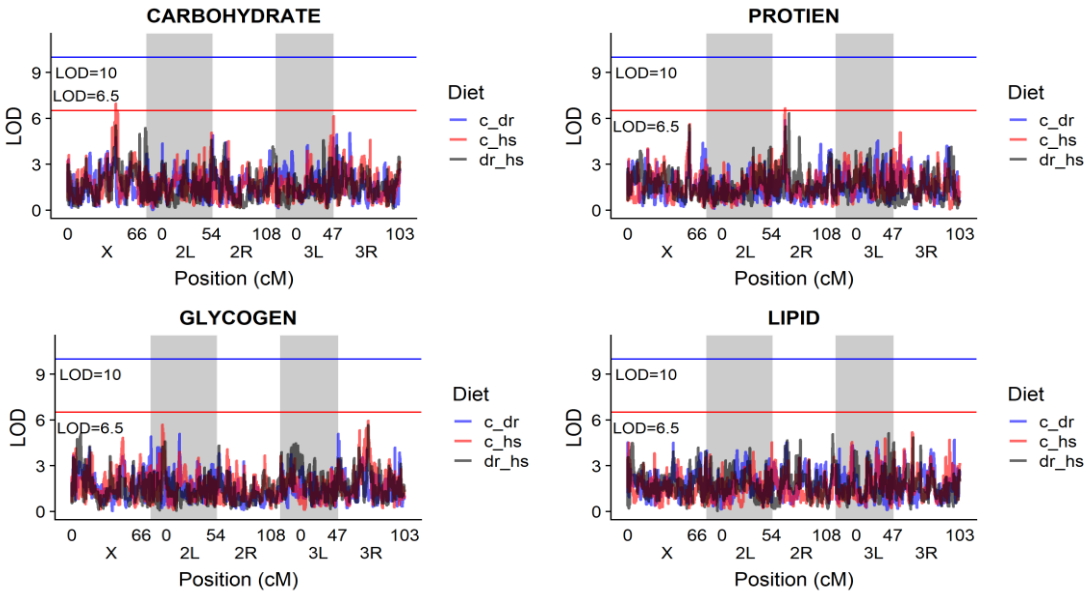


Figure 4: PCA Plot

Below is the PCA plot for Total Acquisition showing the loadings for pc1 and pc2. Red dots are the C; the blue dots are the HS diet and the green dots show the DR diet.

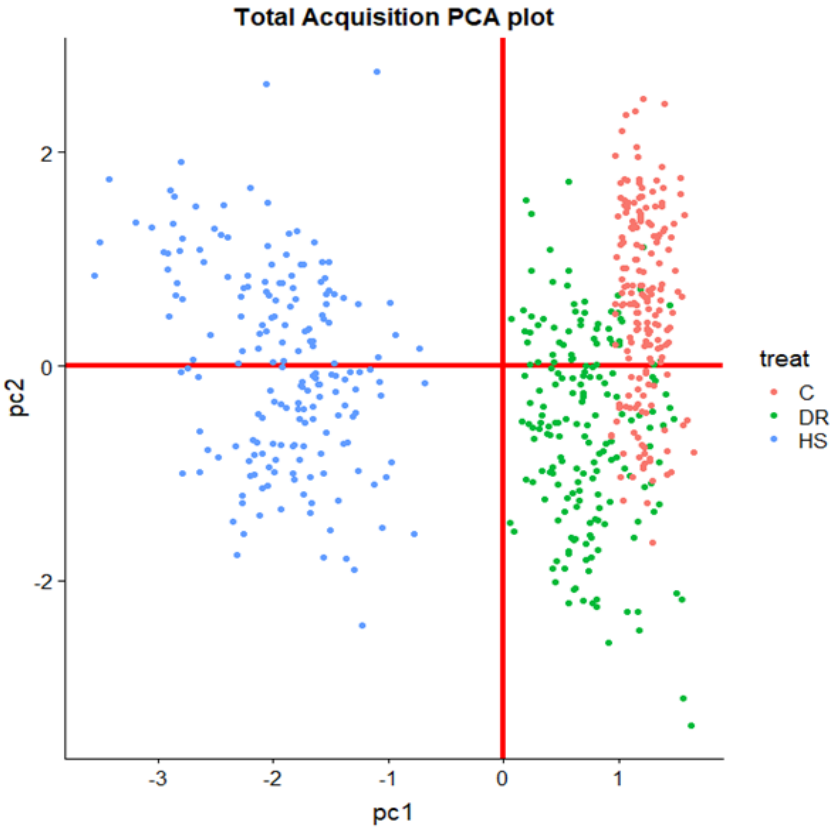


Table 5A. PCA of Total Acquisition of Nutrients

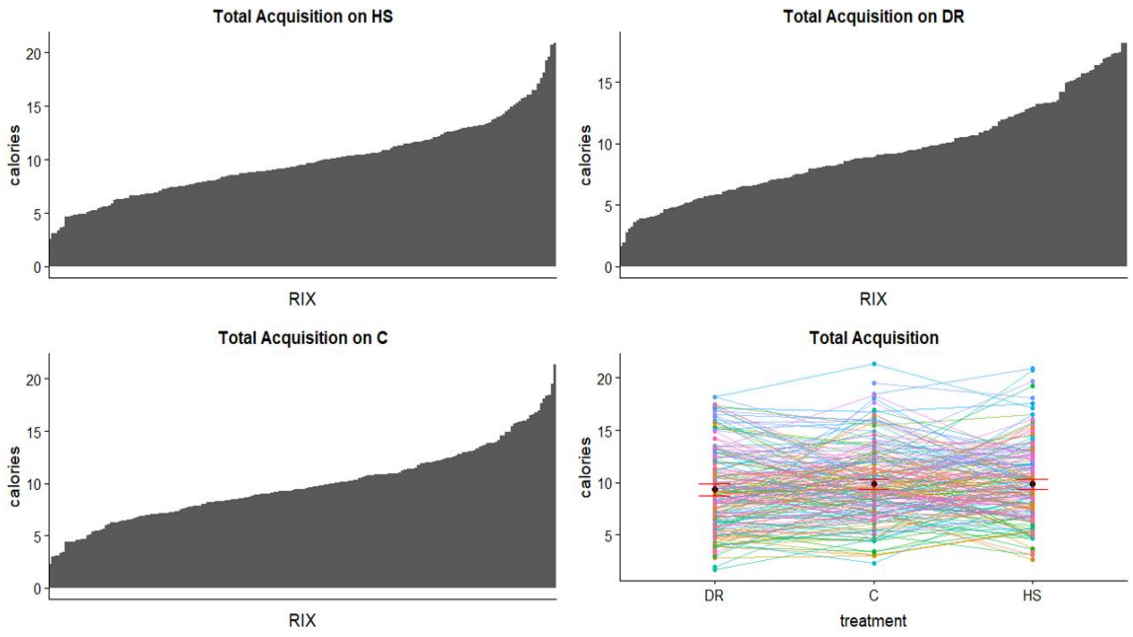
	PC1	**PC2**	**PC3**	**PC4**
Standard Deviation	1.4351	1.0280	0.8810	0.3280
Proportion of Variance	0.5149	0.2642	0.1940	0.0269
Cumulative Proportion	0.5149	0.7791	0.9731	1.000

Table 5B. PCA loadings

	PC1	PC2	PC3	PC4
Carbohydrate	0.6729575	-0.0416616	-0.100619	-0.73162034
Lipid	0.6113909	-0.2064901	-0.427712	0.632949713
Protein	-0.4161313	-0.3982449	-0.777258	-0.25319137
Glycogen	-0.0128136	0.89276375	-0.450342	-0.00068861

Figure 5: Total acquisition

The first three plots depict the total amount of energy acquired (in calories) by RIX on different diets. DR is Dietary Restriction; C is Control; and HS is High Sugar diet. The bottom right plot depicts the total acquisition of all three diets by RIXs. Different line colors indicate different RIXs on the three diets. Mean indicated by black dots and standard error indicated by red bars.



Discussion

In this experiment, we attempted to measure how different nutrient treatments impacted overall fly composition. We measured the effects of a low calorie, low yeast food versus a high calorie, high sugar food on macromolecule amount in the fly. By employing the energy budget assays, we demonstrate that there is variation in phenotype in response to diet. In terms of total acquisition, amount of energy acquired generally scaled up according to diet composition, with DR having the lowest amount and HS having the highest. The exception to this was glycogen. Further, lipid and carbohydrate amounts correlated together on the control diet. Protein consistently correlated with all other macromolecules between .2 and .3 correlation. These data demonstrate that the genotypes responsible for nutrient content storage are plastic and that there are multiple genomic loci of interest. While there have been other studies measuring just protein or carbohydrates or lipids on flies, individually in response to a variety of diets, this study focused on all three components on the same individual flies. This allows a wider understanding of resource allocation in different environments.

Correlations between macromolecule amounts

We produced correlation plots for each nutrient, comparing pairs of macromolecules. There was little correlation between carbohydrates and proteins (0.1987) or carbohydrates and glycogen (0.014). Given that glycogen is an insoluble form of carbohydrate, the lack of correlation between carbohydrate and glycogen is unexpected. We might expect the same processes in content storage for both and, thus,

that amounts might be correlated (Begon *et al.* 1996). Four possible explanations exist for this. First, and most likely given the genome scans, different processes are responsible for carbohydrate and glycogen content storage, or they are biosynthesized by different processes (Begon *et al.* 1996; Heier and Kühnlein 2018). Second, different process within the body require glycogen and carbohydrates at different rates (Chippindale et al. 1998). Third, there is a degree of experimental noise present. The effect of experimental noise became apparent when a technical replicate was present within the plate (of 1305 samples present, 1125 had a technical replicate). Values that had an extremely large difference between the two values, specifically outside 3 standard deviations of the mean, were removed, not unlike the outlier criteria.

Effects of nutrient intake

How flies use these nutrients— proteins, lipids, and carbohydrates—and what diet treatment they are on impacts how nutrients are stored. For example, female flies on high protein food have been found to lay more eggs than those that were on low protein diet or lacking protein altogether (Trevitt *et al.* 1988; Andersen *et al.* 2010)**.** Flies will eat more of a less nutrient dense medium to compensate for low nutritional value. At the same time, flies on a high nutrient diet will eat less volume-wise. (Carvalho *et al.* 2005). Many insects are capable of regulating protein and carbohydrate intake to best suit fitness requirements (Tessnow *et al.* 2018). While we did not measure fly consumption by *volume*, these differences in food intake might have affected our results by decreasing the range between extremes as the flies were not eating the same amount across all diets. Additionally, since these diets varied in amount of protein in diet low (DR) versus normal

(C, HS) and varied in amount of carbohydrate in diet normal (DR, C) versus high (HS), if flies were regulating consumption, the flies would not have eaten the same amounts, volume or calorie-wise, on different diets.

Macromolecule acquisition

Lipids acquired on each diet were significantly different from each other: lowest on the C treatment, highest on HS. This amount of phenotypic variation and large degree of G by E interaction is in line with findings by Reed et al. (2010). It is counterintuitive that C has the lowest amount of lipids, since it is the DR diet that has the lowest amount of calories available for consumption. More expected would have been if the DR diet had the smallest amount of lipid acquisition, given the DR treatment has half the amount of protein of the C treatment. This does, however, lead to an interesting possibility that yeast, *drosophila's* main protein source, may be stored as fat at low levels (Kaun *et al.* 2007). Alternatively, possibly flies are storing more lipids rather than depositing them in eggs (Djawdan *et al.* 1996)**.**

The amount of carbohydrates acquired on Dietary Restriction and Control treatment are the same and significantly lower than the carbohydrates acquired on the High Sugar treatment. In a similar pattern, the amount of glycogen acquired on the DR and C treatments was the same and lower than the glycogen content on the HS treatment, though this was not significant. Glycogen, an insoluble form of carbohydrate, not unexpectedly mimics the carbohydrate plot. In fact, Djawdan et al. (1998) found higher levels of both lipid and carbohydrate on fly lines selected for starvation resistance. Based on this, we might expect higher levels of glycogen storage on the DR diet, but our results

do not indicate this is the case. That being said, we do not expect a plastic response to be the same as an evolved response.

Plastic Traits

Plasticity, individual macromolecule content and different types of diet manipulations have all been studied individually in the past in several species, including *D. melanogaster*. For example, Reed et al. (2010) observed higher levels of variation on high fat diets over any other diet composition; we found a similar result. They also noted that G by E interaction variation is just as high as genetic standing variation and is more than variation that occurs from changes in nutritional environment. Skorupa et al. (2008) found that triglyceride content decreased with higher protein diets, while protein content was unaffected by sugar within the diet. Further, they found higher triglycerides in high sugar environments, similar to what we found. (Skorupa *et al.* 2008). De Luca et al. (De Luca *et al.* 2005) found several QTL peaks responsible for triglyceride content storage, but did not manipulate diet; we did not find the same peaks.

Protein acquired on DR versus Control diet are significantly different from each other, with a higher amount of protein acquired on the C diet. Importantly, protein and lipid specifically are important components of egg composition. As these flies were mated, they most certainly laid eggs; this would result in a certain amount of protein lost from within the fly body (Andersen *et al.* 2010)**.**

Quantitative Trait Loci Analysis

No QTL were significant at the False Discovery Rate. However, we can reasonably say there is variation in phenotype in response to diet. The QTL also indicates evidence of nutrition induced plasticity. This is in line with research within the *Drosophila* species *ananassae* and *melanogaster* (Sisodia and Singh 2012; Stanley *et al.* 2017). There are a few reasons that we did not find any QTL with the given FDR, mainly because the LOD score increases with the number of genome scans run. As we ran genome scans for every macromolecule comparing between diets, we required a high enough LOD score, in this case LOD=10, to prevent false positives. The present data gives us an opportunity for further study.

Limitations

We chose ten days on treatment food after reaching adulthood to ensure that being on treatment food made a significant difference from baseline maintenance food, but that the flies were not on that food so long that survivorship became a legitimate concern. This single snapshot as an adult was chosen so we could be certain the female flies were mated before placed in a new environment. *D. melanogaster*, at different life stages, allocate nutrients in different ways. Certain energy reserves stored during the larval stage are restructured during the transition to adult stage (Aguila *et al.* 2007a). Adults also allocate nutrients differently than larva dependent on diet (Pascacio-Villafán *et al.* 2017). Thus, we waited until adulthood to place flies on diet treatment to ensure that changes energy reserve storage occurred during adulthood.

There was also a certain amount of experimental noise present. This was most apparent when a technical replicate was measured with each plate. While outliers were removed, experimental noise was still present between technical and biological replicates.

We estimated repeatability by comparing measurements between technical replicates. Repeatability was low for a few reasons: first, the assay was not sensitive enough for using only 4 flies per sample; second, the assay required further optimization; and third, the assay was overly sensitive to environmental factors. We optimized the assay to improve repeatabilities and accurate sample measurement by doing the following: first, we only measured whole body samples that had 6 flies; second, we further optimized the assay and changed incubation times for solution evaporation to get a more accurate lipid and carbohydrate reading, and vortexed glycogen vials prior to pipetting into microplates for absorbance measurement to increase even measurement; and third, we instituted new cleaning procedures for the glass microplates, which lowered environmental effects. Vortexing the glycogen vials also lowered the incidence rate of assay debris impacting absorbance measurements, the major contributor to outlier samples and very high glycogen readings that were removed in the histogram plots seen in Supplementary Table S4. Given low glycogen repeatability, this may also explain some of the lack for major variation in glycogen acquisition between diets. Experimental noise may account for the lack of expected correlations between macromolecules and narrow margins of significance in the amounts acquired plots. Additionally, the experimental noise, as well as large number of genome scans, would have influence on the number of peaks reaching a significant LOD score.

Future Directions

Future research will allow us to study how organisms respond to constant high nutrient diets (and other nutrient regimes) and whether they will continue to over allocate

nutrients to storage similarly over several generations. A reasonable second step in this research would be to use the energy budget assays to measure how resources were allocated toward reproductive (using ovarian tissue) and somatic maintenance (using the remaining somatic tissue). Additionally, in order to understand how organisms might respond to different dietary regimes over several generations, we will need to understand the pattern of heredity macromolecule content in different diets and nutrient allocation.

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Supplemental

Conversion Equation

$$amount\ (\mu g) = \frac{total\ concentration\ per\ well\ x\ \frac{volume\ of\ solution\ used\ for\ individual\ assay}{total\ solution\ volume}}{number\ of\ flies\ in\ sample}$$

Table S1. Diet recipes used in experiment.

	Maintenance	DR	C	HS
Water (ml)	1066	1000	1000	1000
Agar (g)	6.25	10	10	10
Dextrose (g)	86.26	-	-	-
Sucrose (g)	-	50	50	342
Molarity	-	.15	.15	1
Yeast (g)	21.6	100	200	200
Cornmeal (g)	40.8	-	-	-
Tegosept (g)	1.8	2.7	2.7	2.7
Ethanol (ml)	7.3	11	11	11

Table S2. Repeatability Estimates

	Protein	Carbohydrate	Lipid	Glycogen
Repeatability	69.57%	26.32%	57.65%	16.29%

Figure S1. Highlighted outliers in macromolecule plots

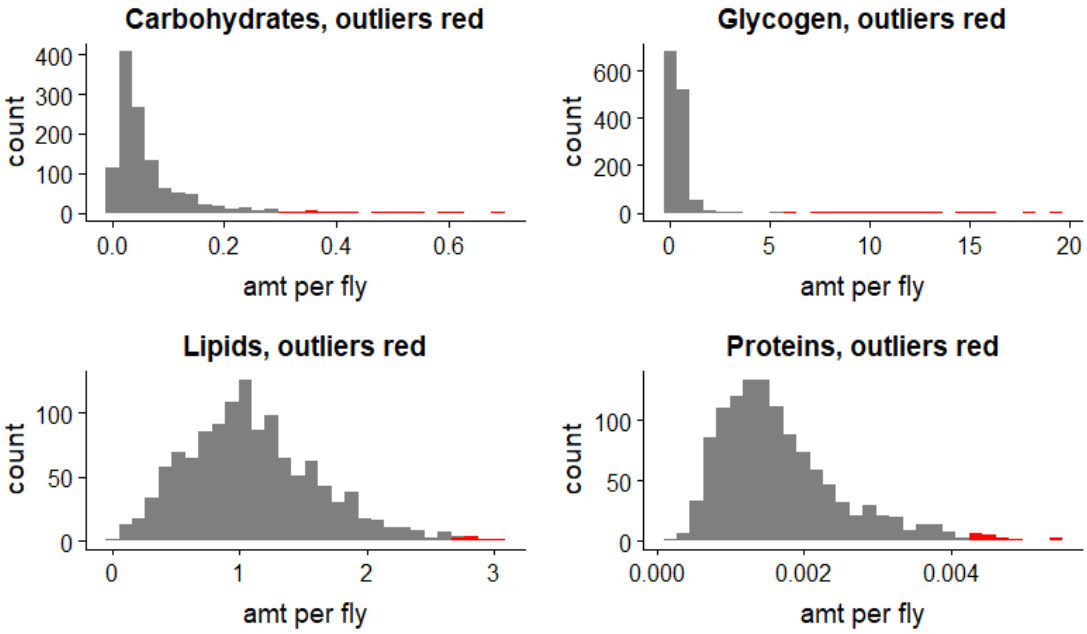


Figure S2. Transformed and untransformed data

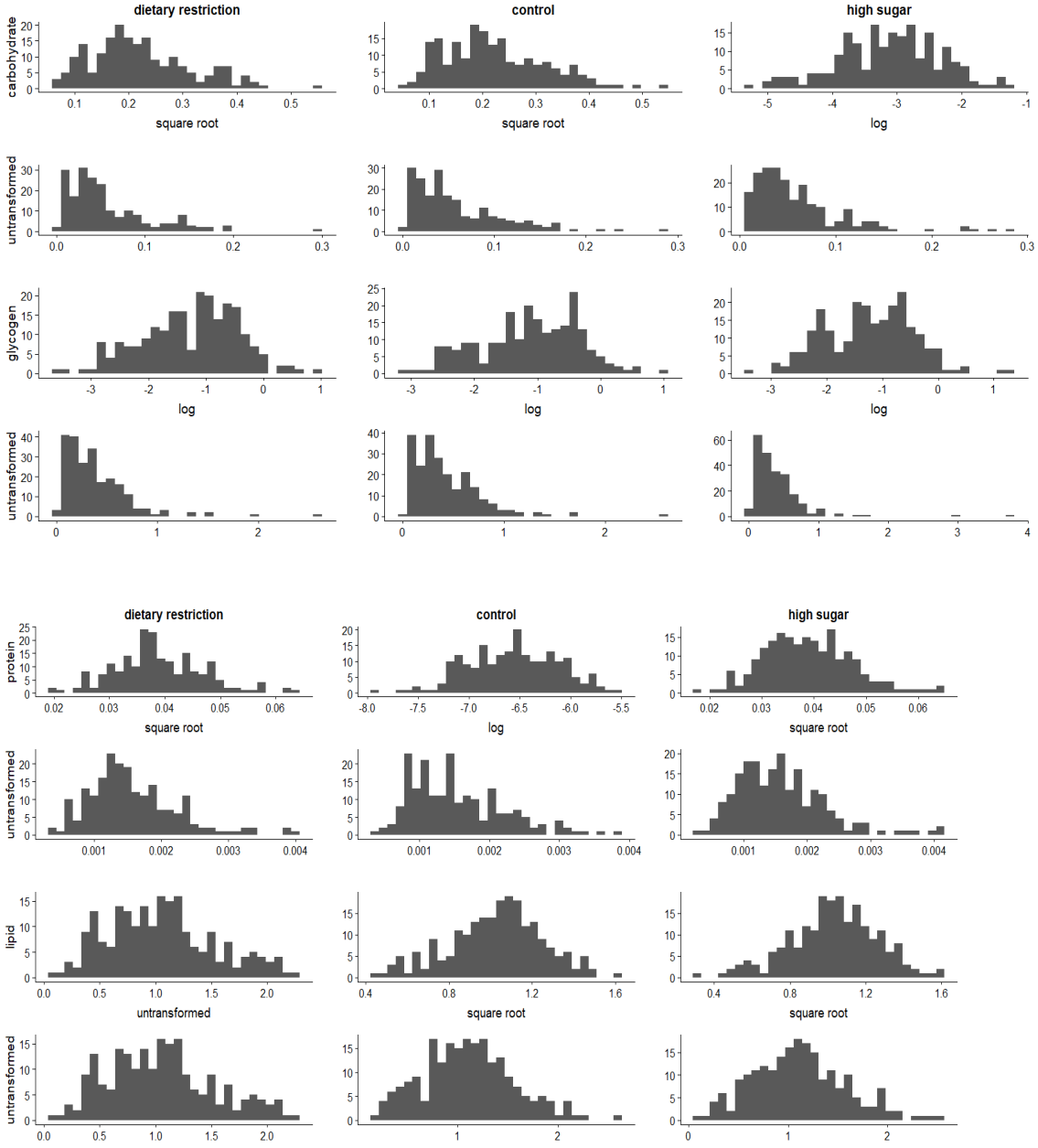


Figure S3. Protein corrected transformed data

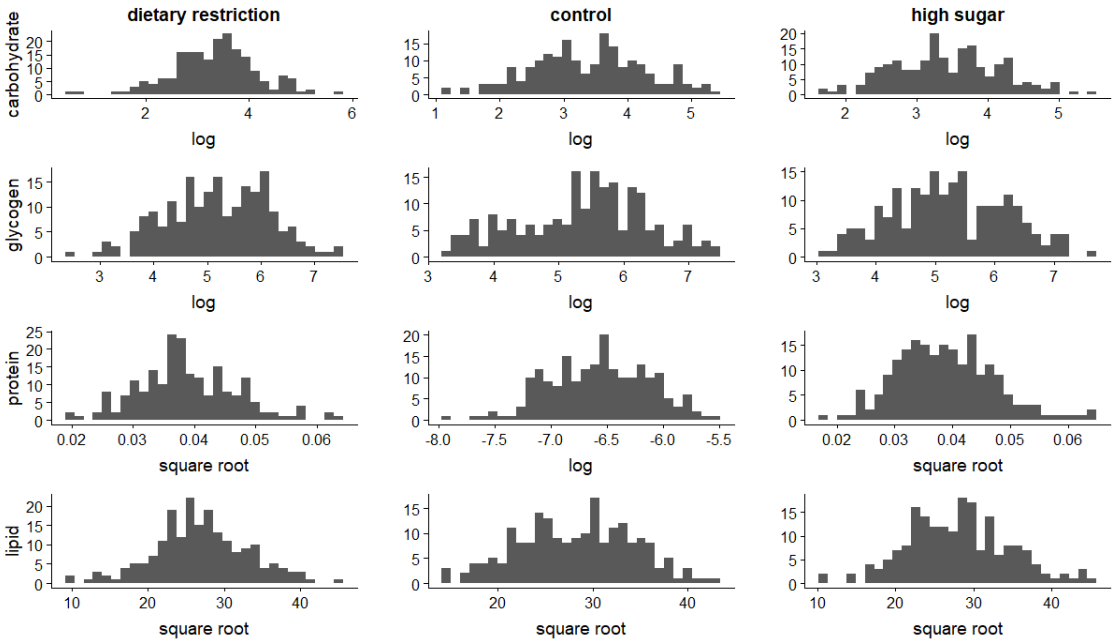
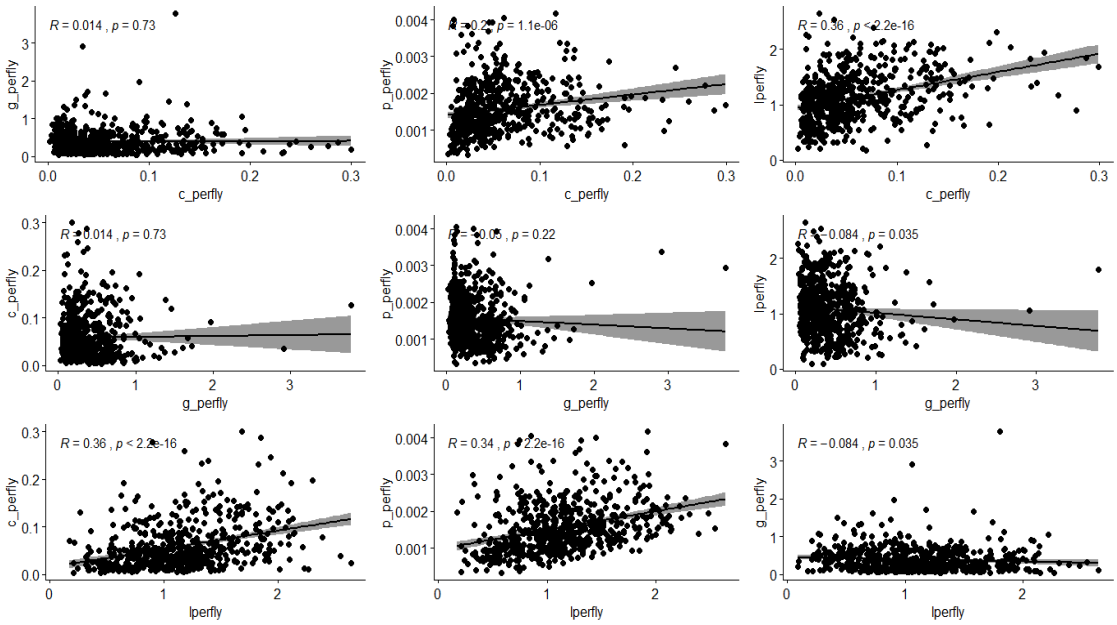


Figure S4. Correlation plots.



CHAPTER 3: Pattern of heredity of carbohydrate, lipid and protein contents in different nutritional environments

Abstract

In order to survive in a changing environment, organisms need to adapt. An organism consumes and stores a finite amount of resources that it uses for all daily tasks. These finite resources must be allocated to different life history traits like reproduction or somatic growth. We used a half sibling population derived from the DSPR, the *Drosophila* Synthetic Population Resource, to understand the pattern of heredity of carbohydrate, lipid, and protein content in different diets and nutrient allocation in *Drosophila melanogaster*.

We generated a half sibling population by mixing RILs from the DSPR for 5 generations and then set up a half-sibling family design. To generate a family we rotated a single male fly (sire) among three different dams every two days. This process was repeated with 25 males with a total of 75 females. The adult offspring of these crosses were placed onto one of three diets for 10 days: a high sugar, low yeast or control diet. We measured energy budget components for the total fly body. We also estimated narrow sense heritability for energy budget components.

Introduction

An organism's nutrition fundamentally affects nearly every aspect of its life: especially in what nutritional resources the organisms will have to use for survival, reproduction, and somatic maintenance. However, an organism in one nutritional environment may allocate nutrients differently when in other nutritional environments. This phenomenon is known as plasticity. Plasticity is useful because it allows adaptation

to different environments increasing the probability of thriving in that environment. Plasticity is most relevant at the population-level, if it is heritable; otherwise, there is little potential for the evolution of a plastic resource allocation strategy.

The leading hypothesis for the evolution of plasticity suggests it evolved as an adaptation to environmental changes (Ho and Zhang 2018; Rago *et al.* 2019). Plasticity in nutrient allocation is a useful strategy for dealing with changing nutrient environments, a situation organisms of many species encounter (Wigglesworth 1949; Stearns 1989; Sgro and Hoffmann 2004; Ng'oma *et al.* 2017). In all organisms, useable energy is stored in three major macromolecules: proteins, carbohydrates, and lipids—otherwise known as an organism's energy budget. Thus, we would expect to see a variation in macromolecule content proportions in different nutritional environments for the same genotype (Lee *et al.* 2008; Aguila *et al.* 2013; Mason *et al.* 2016). For example, an organism on a high sugar diet may devote more nutrients to lipid content, while the same genotype on a dietary restriction diet will devote nutrients to glycogen or protein content instead. Phenotypic plasticity and tradeoffs evolve in resource allocation (van Noordwijk and de Jong 1986; Roff *et al.* 2002a; King and Roff 2010; Shoval *et al.* 2012; Villamarín *et al.* 2016).

We expect plasticity and macromolecule content to have evolutionary potential; offspring of a certain genotype would have the ability to be flexible between different environments and acclimate appropriately to their environments (King and Roff 2010). We have some evidence of organisms switching allocation strategies due to limited resources: rotifers that allocate resources to growth versus reproduction (Stelzer 2001); freshwater killifish that, in low resources, allocate nutrients to survival versus

reproduction (Vrtílek and Reichard 2015); shortlived caddisflies that allocated resources to storage versus reproduction during times of low resources (Stevens *et al.* 2000); and of course, there are numerous examples within *Drosophila melanogaster* where nutrients were allocated to survival versus reproduction (Chippindale *et al.* 1993; Djawdan *et al.* 1996; Flatt *et al.* 2008). However, limited plasticity within a phenotype means that organisms are less able to respond and acclimate to a variety of environments and are only able to survive in one. While plasticity has been noted across a variety of organisms, we still need to determine how heritable plasticity and macromolecule content. Blanckenhorn (1998) found heritable variation in phenotypic plasticity in the growth and body size of the yellow dung fly. Chown et al (2009), Gebhardt and Stearns (1992), Li et al. (2006), and Hoffman et al. (2005) have found evidence of heritability of plasticity in *Drosophila.*

Here, we measure heritability using a half-sibling experimental design set-up. This allows us to measure our traits of interest in both parents and offspring. Specifically, we are interested in narrow sense heritability: $h^2 = V_A/V_P$ where V_A is additive variance and V_P is phenotypic variance. Mapping populations are beneficial because they allow us to find specific regions of interest within the genome, yet are not informative in terms of whether or not a specific trait will be passed on and to what degree. GWAS would possibly let us know if there is a specific SNP for a trait, but since this is a trait for which we expect several genes of small effect on the trait, we would expect a multitude of SNPs (King *et al.* 2014; Lafuente *et al.* 2018). With a half sibling design we can measure how heritable these traits are and how much we can expect to be passed on to the next generation (Bubliy *et al.* 2000) without needing to localize the specific causative variants.

In this paper, we utilize a half sibling experimental design to measure the effects of different nutritional diets on macromolecule content. We fed the adult offspring of a half sibling experimental design set up one of three different diets—a high sugar, a control, and a dietary restriction (low yeast) diet—and evaluated the effects of these diets with a modified Foray et al's (2012) assay that measured lipids, proteins, soluble carbohydrates and glycogen, an insoluble form of carbohydrates. Since we used flies from half sibling and full sibling families, we were able to study the heritability of these traits. In this paper, we:

1. Measure macromolecule content across full and half sibling families across 3 different diets.
2. Establish the evolutionary potential of how macromolecule content responds to differences in nutritional environment.
3. Measure the variation, plasticity and genetic correlation between diets present in these flies.

Methods

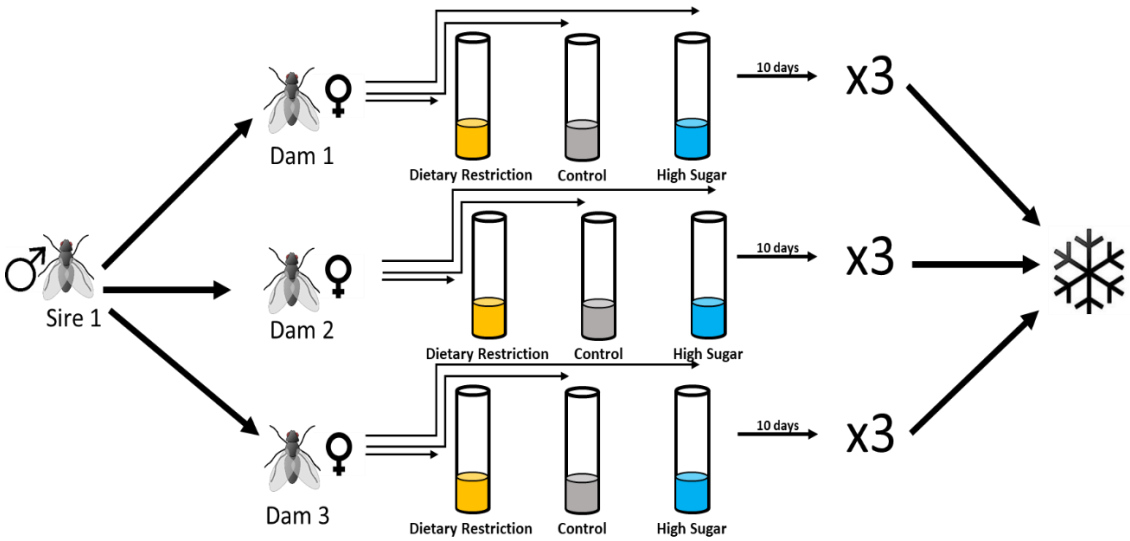
Fly Husbandry & Experimental Population

We generated a synthetic population from the DSPR. The *Drosophila* Synthetic Population Resource (DSPR) is a multiparent mapping population comprised of 2 separate populations: an A and B population. Two sets of fully sequenced founder lines (A1-A7 and B1-B7 plus a common founder line for both, AB8) were interbred for 50 generations. The ensuing RILs were then inbred for 25 generations to produce a total of over 1500 Recombinant Inbred Lines or RILs between the two populations (flyrils.org; (King *et al.* 2012a; b). More information about the DSPR, including its generation, properties and statistics can be found in King et al (King *et al.* 2012a; b) and at http://FlyRILS.org.

To generate a synthetic population from the DSPR, we used 5 random females per RIL from 835 DSPR B population RILs, and placed them into 6 cages with maintenance diet food. Over the next two days, we collected eggs (22-hours oviposition) and placed them in 250ml glass bottles. We released adult flies from the glass bottles to the 6 cages and allowed them to mate for 5 generations. Populations in the cages were larger than 2000 flies per cage. We fed the population via a Petri dish in each cage containing maintenance food and watered them with a micro-petri dish filled with cotton wool moistened with water. Food dishes were exchanged 3 times weekly. For the following generations, we collected eggs via very thin slices of food off the petri dish and transferred these to 30 vials per cage. Each vial had approximately 50-90 eggs. These vials of eggs were dispersed across the 6 cages to produce a genetically homogeneous

population. The five generations were on 3-week cycles (oviposition to egg collection) prior to the start of the half sibling experiment.

Figure 1. Half Sibling Experimental Design Set Up.



To generate a half-sibling family (See Figure 1): we rotated a single male fly (sire) among three different female flies (dams) every two days. We repeated this process with 25 males with a total of 75 females. We aimed to generate a total of six vials per sire-dam pairing so that there would be two vials for each diet treatment: dietary restriction, high sugar and control. We placed 15-day post-oviposition adult female offspring on one of the three treatment diets for 10 days. The female offspring of the sire-dam (S-D) pairings were frozen, then separated into groups between four and six individuals and placed in Eppendorf tubes in a 4˚F freezer awaiting energy budget assay measurement. For every half-sibling family where we had at least 10 offspring of one sire plus the three dams, per dam, we dissected the offspring of one sire-dam pairing (full sibling family). We used mated females for the energy budget assay. Eggs were laid by the females in this assay, but measuring egg number was beyond the scope of this

experiment. Another member of the lab did measure egg number and other life history traits which has been published separately. We removed ovarian tissue from the female fruit flies and stored it; we stored somatic tissue in a separate Eppendorf tube so that we could run the energy budget assay on these tissues separately. This allowed us to measure energy budget components for reproductive tissue and somatic tissue separately while still allowing us to add these together to get the energy budget components for the total fly body. We used identical diet treatments—Control (C), Dietary Restriction (DR) and High Sugar (HS)—to our previous experiment. DR was a calorie restricted, low yeast diet; HS was a diet high in sugar and calories (diets adapted from Bass *et al.* 2007; Skorupa *et al.* 2008). The C diet was scaled between the two, with C and HS diets having the same amount of yeast and the C and DR diets having the same amount of sugar.

Energy Budget Assays:

We used a modified version of Foray et al's ((Foray *et al.* 2012) energy budget assay modified to run in a microplate from Van Handel's (Van Handel 1985; Van Handel and Day 1988) original mosquito assay. We measured proteins, lipids, and carbohydrates in whole female fruit flies, and dissected ovaries and somatic tissue. We homogenized six whole female *Drosophila melanogaster* (or ovaries/ somatic tissue of 10 female fruit flies) with a stainless-steel bed and aqueous lysis buffer solution using a BeadBeater in an Eppendorf tube. We aliquoted 2.5 microliters of the supernatant of this solution in the Bradford assay to measure proteins. We used Bovine Serum Albumin as the standard curve. We added chloroform-methanol and sodium sulfate to the supernatant, centrifuged then measured carbohydrates and lipids from the resulting supernatant and glycogen from

the pellet in borosilicate microplates. We were able to use plastic microplates for the protein assay because no volatile chemicals were used in that assay. Borosilicate plates were used for the three other macromolecules to prevent plastic contamination in the supernatant.

To measure carbohydrates: we evaporated 150 microliters of supernatant in a borosilicate microplate to 10 microliters in a vent hood. Following a recommendation from Cheng et al, we increased the length of evaporation time in the vent to 40 minutes, instead of Foray's 15 minutes (Cheng *et al.* 2011; Foray *et al.* 2012). We added anthrone reagent, incubated it for 15 minutes at room temperature, and then for 15 minutes in a 90 degree C water bath, cooled it, and read the absorbance in a plate reader. We also used anthrone to measure glycogen. We used dextrose in methanol as the standard curve for both soluble carbohydrates and glycogen.

To measure lipids, we pipetted 100 microliters of supernatant and evaporated it in a 90 degree water bath until dry. Next, we added vanillin and sulfuric acid to each well and used a chloroform standard curve to measure absorbance. We ran samples in the same way for dissected tissues.

We used standard curves (a known concentration and total macromolecule amount) to convert sample absorbance to concentration then to amount (See Supplementary Table S1). We used standard curves on each microplate for each macromolecule. We measured absorbances per well. Because each well was a specific proportion of the total solution, we needed to calculate total macromolecule amount per well to include the whole solution and further calculate per fly. Here, we present macromolecule amount in micrograms per fly. Nearly every sample had at least one

technical replicate, which was run concurrently within plate. For statistical analysis, we used the means of these two samples.

Optimizations:

We modified the Foray et al.'s ((Foray *et al.* 2012) energy budget assay that used parasitic wasps, for 6 whole fruit fly bodies, instead. For the following experiment, we optimized the assay for dissected reproductive (ovaries) and somatic tissue. For each of the four assays measuring the macromolecules, we tested a range in number of dissected fruit flies (7 to 14 flies). Eventually, we chose 12 flies to be dissected based on the evidence that that number had the highest replicability. Additionally, to ensure the highest degree of accuracy we also tested two different volumes of solution used to assay for protein. The amount settled on was 2.5 microliters. Additionally, we tested the amount of solution used for the carbohydrate trials—150 microliters and 300 microliters—as well as varying the amount of time for the evaporation step (Cheng *et al.* 2011). 150 microliters of solution evaporated for 80 minutes in a vent hood yielded the best results. (See Table S2, in Supplementary for Repeatability measurements).

Statistical Analysis

All analysis described below were performed in R (R Core Team 2018).

Pre-processing

We removed outliers from this dataset using the following method: we plotted individual macromolecule contents on a histogram and calculated the standard deviation and means for each macromolecule. We identified outliers as any value three standard

deviations from the mean, or more than one standard deviation from its technical replicate. If the value was more than one standard deviation from the technical replicate, both values were eliminated. We split the data into two datasets: one dataset for samples that had 2 technical replicates within the plate; a second dataset for samples that had more than 2 two replicates or biological replicates. For the dataset with 2 technical replicates: if there were samples identified as three standard deviations from the mean but were paired with technical replicate—as in, not a single sample but a pair of technical replicate samples and not farther than 1SD from its technical replicate—they were kept in the dataset. For lipids and proteins, all values more than three standard deviation away from the mean were removed, as those identified with outliers did not have their technical replicate within 1 standard deviation. For carbohydrate and glycogen, we identified values more than 3 standard deviations away from mean and confirmed that they were grouped together by Well.ID (they agreed with replicate and were not removed). For the dataset with more than two technical replicates, we plotted the samples, and any samples more than 3 standard deviations from the mean were removed.

Once the outliers were removed, we found the mean between technical replicates so that each sample had a single value. Then, we combined the two datasets into a single dataset. We then proceeded to check whether the data was normally distributed. Plots were produced for individual macromolecules and further broken down by treatment. Macromolecule contents were then transformed to a normal distribution if they did not already adhere to a normal distribution. The protein and lipid samples remained untransformed. We left untransformed the glycogen content amounts on each treatment, except for the HS treatment which we square rooted to adhere to a normal distribution.

Lastly, all treatments for carbohydrate were logarithm transformed. We transformed data until a normal distribution in order to run statistical tests that required a normal distribution of data.

Amounts acquired plots

We produced plots, measuring amount of macromolecule across three diets, or amounts acquired plots, for each macromolecule using untransformed, absolute values. We took the separated macromolecule datasets and for each sire family and averaged the samples for each diet treatment. We then plotted the mean of each sire family across diet treatments. Colors varied by half-sibling family.

ANOVA

We conducted an Analysis of Variance (ANOVA) to determine the effect of diet treatment on macromolecule amount, using data adhering to a normal distribution. We fit a linear model for macromolecule content of sire family means as modeled by diet treatment. We used the 'lm' function in R to fit the following linear model:

$$Y_{ij} = \mu + \tau_i + \epsilon_{ij}$$

where Y_{ij} is j^{th} observation of response of i^{th} treatment, μ is fixed common effect, τ_i is the effect of treatment, and ε_{ij} is the random error present. We then used 'anova' from base R to measure the effect of diet treatment on macromolecule. We followed up with Tukey tests to account for the multiple tests performed.

Animal Model

We used the animal model to estimate narrow sense heritability (h^2); where V_a is the additive variance and V_p is the phenotypic variance.

$$h^2 = V_a / V_p$$

We modified a procedure as described in Ng'oma et al (2017) (and originally described by: Kruuk 2004; Wilson et al. 2010; Ingleby et al. 2013) detailed below. Using the dam data from the samples, we created a pedigree indicating the sire and dam of each offspring. Additionally, as we were using the R package, *MCMCglmm* (Hadfield 2010), we indicated that we did not have data for the parents of the sire and dam parents. For the model, we used 2,000,000 iterations and 15000 iterations as burnin. We thinned every 50 iterations. We used identical priors to Ng'oma et al (2017), where V = 1 and nu = 0.002 (weakly informative). In this way, we were able to produce, for each macromolecule, density plots of the estimated posterior probabilities— posterior probabilities is a Bayesian statistical term denoting the probability of a hypothesis after experimental observation (Lee)— for heritability across diet treatments.

Results

Phenotypic Patterns

We measured the amount of macromolecules—specifically protein, lipid, soluble carbohydrates and glycogen—acquired across 3 different diets. We fit a linear model using transformed amounts per fly modeled by diet for each macromolecule. We performed separate ANOVAs (Analysis of Variance) for each macromolecule and followed up with Tukey tests. For amount of carbohydrates acquired, HS varied significantly from C and DR. There were low amounts of carbohydrate acquired on both DR and C, and then drastically more carbohydrates acquired on the HS diet (Figure 2).The amount of lipids acquired across all three treatments were significant between treatments (Table 1). The lowest amount of lipid was acquired on the C diet, and the highest amount of lipid was acquired on the HS diet. The opposite was true for the protein plot. We can see that for some families, the amount of proteins gradually scaled up as calories increased. For other families, the highest amount of protein was acquired on the C diet with less acquired on the DR and HS diet. However, overall, for the means per treatment, the lowest amount of protein acquired on DR and the highest on the C diet. Tukey tests run on protein acquisition indicate that the amount of protein acquired on HS and C treatment only minorly differed from each other and that DR was significantly different from C and HS. Glycogen, in a similar pattern to lipid, had the smallest amount acquired on the C diet and then DR and HS respectively. The amount of glycogen acquired on the DR, HS and C diets differed significantly from each other (Table 2) There is a great deal of variation in response to diet, and especially, between and within families.

The methods used to assay amounts of proteins, lipids, carbohydrates, and glycogen does not distinguish between absorbed and unabsorbed food material within the fly body. For example, DR treatment food has half the protein as the C or HS diets, and HS has over 6 times the amount of sugar (or carbohydrate) as the DR or C diets. The assay would pick up this unabsorbed food in the gut as well as overall macromolecule amount throughout the body. However, the food in the GI tract would eventually be absorbed as fuel and the amounts in the GI tract are minor compared to the fly body so we will continue under the assumption that the results of this experiment are mainly absorbed and assimilated nutrients.

Heritability

We estimated the heritabilities of each macromolecule (protein, lipid, carbohydrate and glycogen) on each diet (C, DR, and HS). Our heritability estimates for carbohydrate, protein, and glycogen on each diet ranged from 0.31-0.58. A table of our results is shown below (Table 3 & Figure 3). 'Lower' and 'Upper' indicate the lower and upper values (credible intervals) of the 95% highest posterior density (HPDI). There is a figure showing sampling in Supplement (See Supplementary Figure S1)

Genetic Correlations

Additionally, we wanted to measure if there was any gene by environment interaction. Significant G x E is indicated when genetic correlations between environments is less than one. We found, for each macromolecule in pairwise diet comparisons, genetic correlations less than one (see Figure 4). For specific values of genetic correlations and HPDI's, see Table 4.

Figure 2. Effects of Diet on Energy Budget in Half Sibling Families

Diet is on the x axis, amount of macromolecule acquired in micrograms on the y axis. Black dots indicate diet means and red lines indicate error bars. Colored lines are half sibling families.

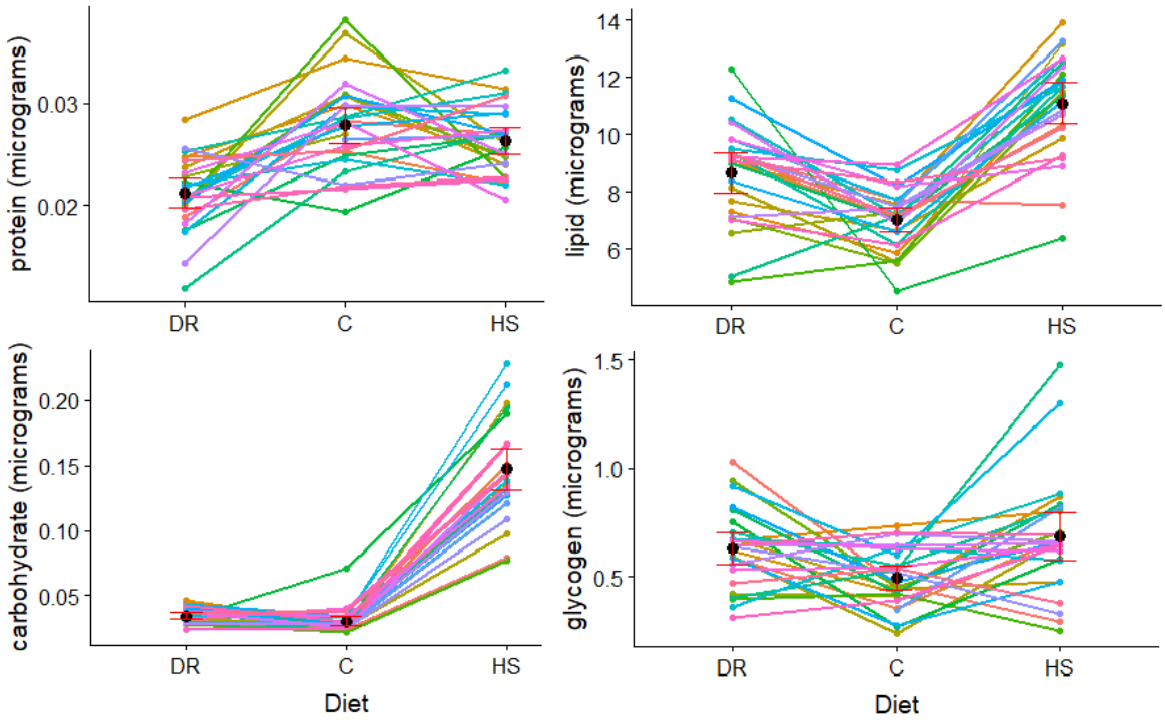


Table 1. Means and SE of Amounts acquired plots

	DR	DR	C	C	HS	HS
	Mean	SE	Mean	SE	Mean	SE
Carbohydrate	0.03451	0.001024	0.03038	0.001421	0.1475	0.004771
Lipid	8.7169	0.17836	7.0441	0.14196	10.9473	0.2578
Protein	2.1235e-2	4.0298e-4	2.778e-2	5.0136e-4	2.586e-2	5.042e-4
Glycogen	0.6425	0.02496	0.5114	0.01944	0.6649	0.03123

Table 2. Analysis of variance for macromolecule amount

Source refers to macromolecule, df is the abbreviations for degrees of freedom, MS stands for Mean Square, P indicates significance for protein, lipid, glycogen and carbohydrates.

Source	**df**	**SS**	**MS**	**F**	**P**
Carbohydrates	2	139.986	19.9929	372.2	< 2.2e-16***
Total	76	4.082	.0537		
Lipid	2	222.12	111.061	46.97	5.245e-14***
Total	76	179.70	2.365		
Protein	2	6.436e-4	3.218e-4	21.771	3.351e-8***
Total	76	.0011234	1.478e-05		
Glycogen	2	1.0949	.54745	23.802	9.412e-9***
Total	76	1.748	0.023		

Table 3. Heritability estimates and 95% HPDI for each treatment and macromolecule

	HS			DR			C		
	h^2	**Lower**	**Upper**	**h^2**	**Lower**	**Upper**	**h^2**	**Lower**	**Upper**
Glycogen	0.5852	0.2918	0.9718	0.3148	0.04166	0.6303	0.3327	0.0718	0.6433
Protein	0.5394	0.4347	0.6444	0.5499	0.4562	0.641	0.5381	0.4337	0.6451
Carbohydrate	0.4601	0.1697	0.8013	0.435	0.1224	0.801	0.3325	0.0709	0.6431
Lipid	0.8344	0.3535	0.999	0.9175	0.578	0.9999	0.2247	8.29E-05	0.4486

Table 4. Genetic correlations and HPDI pairwise diet comparisons.

	STD vs HS			STD vs DR			DR vs HS		
	Median	Lower	Upper	Median	Lower	Upper	Median	Lower	Upper
Glycogen	0.7945	0.251	0.9993	0.1965	-0.6961	0.9984	0.2827	-0.4925	0.9988
Protien	0.7306	-0.2924	0.9996	-0.07097	-0.7735	0.5665	-0.00495	-0.8281	0.9197
Carb	0.2381	-0.3879	0.7594	0.5062	-0.3059	0.9699	0.1665	-0.4622	0.7246
Lipid	0.4225	-0.3687	0.9966	0.6376	0.1097	0.9975	-0.3054	-0.9024	0.3104

Figure 3. Density plots of the estimated posterior probabilities for each macromolecule across diet.

Lavender indicates the C diet, DR is shown in pink, and HS is shown in green.

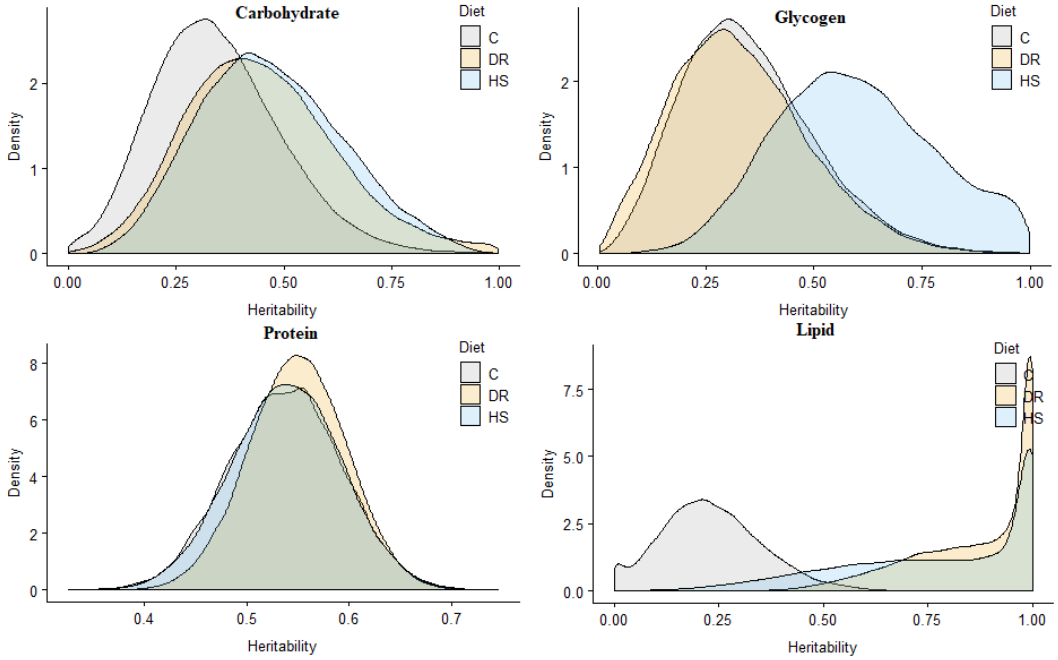
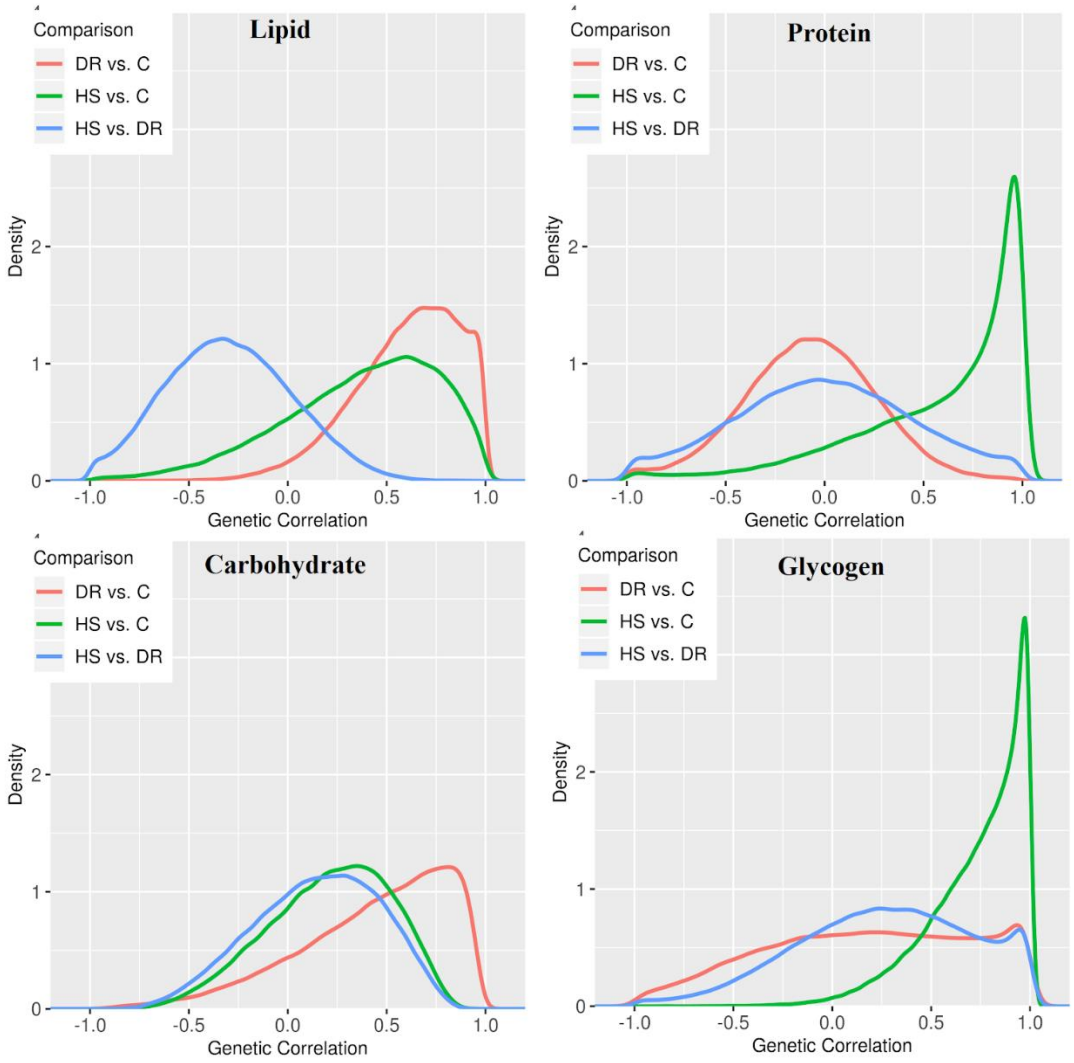


Figure 4. Density plots of the estimated posterior probabilities for the genetic correlation between macromolecules in pairs of diets.

Red lines indicate the comparison between the DR and C diet. The green lines show the comparison between the HS and C diet. Blue lines show the difference between the HS and DR diet.



Discussion

In this study, we used a half-sibling design experiment to measure heritability estimates for lipid, carbohydrate, glycogen, and protein contents across three different diets. Our results show differing heritability for different macromolecule contents across nutritional environments, suggesting nutrient content changes based on the particular environment of a genotype. While there are some examples of this phenomenon in literature (Harrison *et al.* 2007; Moreira *et al.* 2012; Sisodia and Singh 2012), only a few measure all four macromolecules across a low and high calorie diet to measure heritability.

Phenotypic Patterns

We evaluated the effects of three nutritional environments on the allocation of nutrients to the following macromolecule types: protein, lipid, soluble carbohydrate, and glycogen. Based on the nutritional environment, the amount of nutrients stored in the body as lipids, carbohydrates, or proteins will change even as the total amount of nutrients acquired changes (Wigglesworth 1949; Sgro and Hoffmann 2004)**.** Acquisition and allocation are known to vary among individuals and are influenced by both genetic and environmental factors (Chippindale *et al.* 1998; Reed *et al.* 2010b). *D. melanogaster*, at different life stages, allocate nutrients in different ways. Certain energy reserves stored during the larval stage are restructured during the transition to adult stage (Aguila *et al.* 2007a). Adults versus larva also allocate nutrients differently dependent on diet (Pascacio-Villafán *et al.* 2016). Thus, we waited until adulthood to place flies on diet treatment, to ensure that changes energy reserve storage occurred during adulthood.

We might expect that glycogen and soluble carbohydrates, as they are both different forms of the broader macromolecule carbohydrate (glycogen is an insoluble form), would have similar patterns of acquisition. This does not appear to be the case. We can see, first, in carbohydrate that low amounts are acquired in both the DR and C diets and drastically more on the HS diet. This pattern is the same across families. On the other hand, for glycogen, acquisition means are the lowest on the C diet and HS and DR have similarly higher amounts. Further, glycogen does not have a similar pattern across families. While *most* families follow the pattern described above, several had significantly less variation and some families acquired glycogen in a completely different pattern, most commonly, the highest amount on the C or DR diet. This suggests different mechanisms for carbohydrate and glycogen acquisition. Additionally, there may be a single or agreeing mechanisms for carbohydrate allocation across 3 different diet treatments. This does not appear to be the case for glycogen.

For protein, we found that the highest amount acquired was on the C diet and the lowest on the DR diet. This is not entirely unexpected given that the DR diet has half the nutritional protein source (yeast) available within the diet. Generally, families follow a low DR protein acquisition and a high protein acquisition on C diet. However, some families have moderately level amounts of protein acquired across all three diets. This suggests that while there is a general trend, there are different mechanisms at play for protein acquisition across all three diets.

For lipid, we found that the highest amount acquired was on the HS diet, and the lowest on the C diet. It is unsurprising that HS yielded the highest lipid acquisition. However, more lipid acquisition on the DR diet vs the C diet was not expected given that

the DR diet has fewer calories than the C diet. Overall, there are four families that do not fit this pattern, but the vast majority do. However, credible intervals overlapped between diets for protein, carbohydrate and lipid. Lipid, especially, had a wide credible interval. Glycogen, alone, had credible intervals that did not overlap.

In Chapter 2, we measured energy budget components in the RIL's of the DSPR as well as half and full sibling families from a base population created by mixing DSPR RIL's. We found strong patterns of carbohydrate acquisition in our heritability experiment that we did not see in the RIL's; flies from the heritability experiment also stored vastly higher amounts of lipids. Lipid acquisition in the heritability experiment showed the trend of highest acquisition in HS and lowest acquisition on C food. The range of glycogen acquisition in the RIL's was broader than glycogen acquisition in the heritability experiment. Lastly, we saw strong patterns of protein acquisition in the heritability experiment absent in Chapter 2.

Heritability and Genetic Correlations

While previous studies have looked at heritability of lifespan, fecundity, wing-type, and ethanol resistance in *D. melanogaster* (Hoffmann and McKechnie 1991; Bubliy *et al.* 2000; King *et al.* 2011; Ng'oma *et al.* 2017), few have looked at heritability of macromolecule content within the same fly across several diets, with Reed et al.'s paper (2010) being the exception. However, their paper mainly focused on heritability in a single diet, and macromolecule content was studied within a nutritional geometry context and included a high fat diet. Clark et al (1990) looked at the heritabilities and effects of selection on lipid and glycogen content in *Drosophila.* Scheitz et al (2013) looked at heritability of specific lipid molecules but not overall content. Lipid heritability in

humans is also of strong research interest (Heiberg 1974; Goode *et al.* 2007; Wang *et al.* 2009; Zarkesh *et al.* 2012). Here, while credible intervals overlapped between diets for protein, carbohydrate and lipid; lipid especially had a wide credible interval. Glycogen alone had credible intervals that did not overlap.

A review by Mackay (2001) and a model by De Jong (1990) and experiments by several others declare that different genetic loci are responsible for differing responses to environmental changes (Sgro and Hoffmann 2004). Indeed, different loci are responsible for differing responses to changes in nutrient diet (Reznick, Nunney, and Tessier 2000). It is suggested that given different loci are responsible for the energy components (protein, carbohydrate and lipid), we can anticipate that heritability will vary among these phenotypes (Sgro and Hoffmann 2004). Our study agrees with these findings.

Limitations

Resemblance between offspring and parents is indicative of heritable traits. However, heredity is not the only reason offspring might resemble their parents. For example, if parents and offspring exist in the same environment, they will likely be impacted in similar ways. We know maternal effects and environmental effects can impact phenotype, so we try to combat this by raising parents on maintenance food and only using treatment food for adult offspring (Dew-Budd *et al.* 2016). An alternative is that phenotype rather than genotype influences the offspring phenotype, for example, if a parent is able to acquire more food for their offspring (Roff *et al.* 2002a). This is less likely in our lab raised populations where we maintain population size. Finally, sample size is another limitation, a problem common to the vast majority of quantitative genetics experiments.

Between Chapter 2 and 3, methodologies were mainly the same, in that we took adult female flies and put them on one of three different diets for 10 days and after 10 days on experimental food they were flash frozen and put through the assay. However, the main difference between the two experiments was the origin of the flies we used. For Chapter 2 flies, we used DSRP Recombinant Inbred Lines crossed with a DSPR founder line, so we knew there was only one of 8 possible haplotypes possible at each location. This allowed us to be able to QTL map the phenotypes we were interested in. For the Chapter 3 flies, while we still used flies from the DSPR mapping population, they had been allowed to randomly mate for 5 generations, so we were no longer using inbred lines. This intercrossing also meant we were no longer able to QTL map. However, we used these flies to generate a half sibling population which did allow us to estimate narrow sense heritability.

Conclusion

In conclusion, in our experiment we showed that macromolecule content changed across different diets and differing heritability for macromolecule content across nutritional environments. Heritability tells us broadly about the genetics but not the specific loci responsible for macromolecule contents. Calculating heritability allows us to predict how a population will respond to selection (Zimmer and Emlen 2016). In *D. melanogaster*, diet alone can account for 1 to 2% of variation in metabolic traits. Genetics can account for between 11 and 23% variation in metabolic traits. However, most interestingly the interaction between genotype and diet can account for 12 to 17% variation (Reed *et al.* 2010b). Our findings lead to an interesting question: does parental

nutrient availability change how offspring adapt and respond to different nutritional environments? A study examining the effects of diet over several generations would be especially fruitful for confirming the heritability estimates of macromolecule content.

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Supplemental

Table S1. Standard Curves

Concentration /Dilution	Example absorbance	Lipid amount
1.5	0.139	200
1.3	0.155	175
1.1	0.157	150
0.9	0.129	125
0.7	0.099	100
0.5	0.105	75
0.3	0.065	50
0.1	0.041	25

Concentration /Dilution	Example Absorbance	Protein amount
3	0.254	3
2	0.189	2
1.5	0.053	1.5
1	0.0375	1
0.75	0.014	0.75
0.5	0.016	0.5
0.25	0.004	0.25
0.125	-0.002	0.125

Concentration /Dilution	Example Absorbance	Glycogen amount
24	2.474	75
21	2.207	65
18	2.065	55
15	1.723	45
12	1.339	35
9	1.004	25
6	0.59	15
3	0.189	5

Concentration/ Dilution	Example Absorbance	Carbohydrate amount
0.25	-0.002	0.125
0.5	0	0.25
0.75	0.001	0.5
1	0.023	1
2	0.1	2
3	0.158	3
4	0.222	4
5	0.271	5

Supplementary Figure S1

Heritability Sampling: Lipid

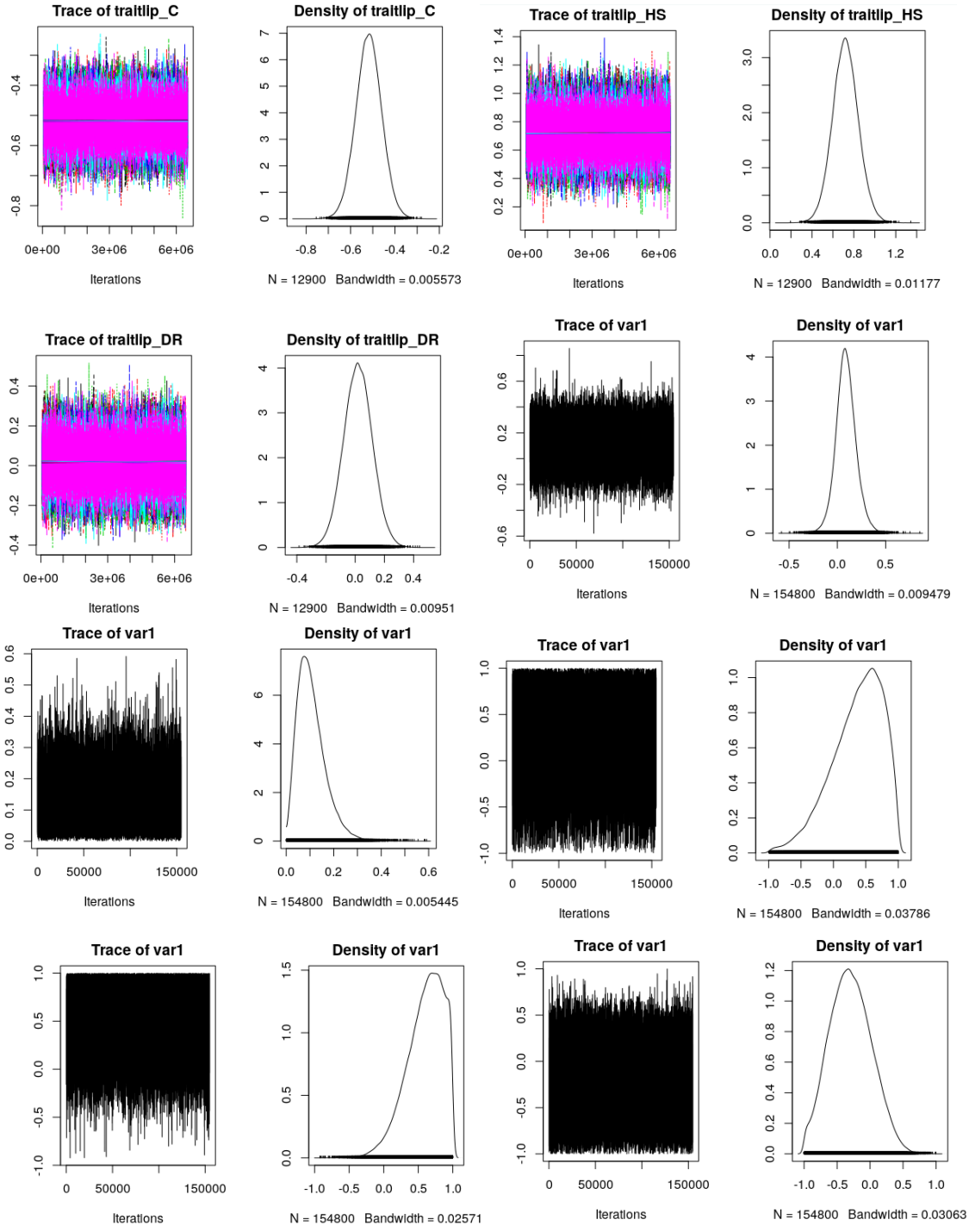


Table S2. Repeatability measurements

	Carbohydrate	Glycogen	Protein	Lipid
Repeatability	95	93	37	83

CHAPTER 4: Effects of diet-based selection on macromolecule content in *Drosophila Melanogaster*

Abstract

Organisms must survive in a variety of nutrient environments. In order to do so, they must be able to adapt to their environment. Organisms have a finite energy budget, or source of nutrients, they may draw upon to use for all daily tasks. These finite resources will be differently allocated to reproduction and somatic growth. We used a mixed population derived from the DSPR, the *Drosophila* Synthetic Population Resource, to understand the evolutionary response due to selection. We studied carbohydrate, lipid, and protein content allocated to reproductive tissue and somatic tissue on different diets and different selection treatments in *Drosophila melanogaster*.

We placed flies on one of three different nutrient treatment selection regimes for 30 generations: a deteriorating availability of nutrients, a fluctuating availability of nutrients, and a constant high availability of nutrients. After undergoing selection, at Generation 30, we took the adult females of each selection line and set them on one of three different food treatments for 10 days. After 10 days on this food, we dissected the ovaries, and ran the energy budget assay separately on the somatic tissue and the reproductive tissue. We summed these values to get the "whole body" macromolecule content measurement for energy budget.

Introduction

In the last several decades, obesity has been on the rise in the United States and globally (Satoh-Asahara *et al.* 2015). Humans are naturally predisposed towards obesity because they tend to store nutrients in high resource environments. However, this tendency towards storage varies across different nutritional environments and among individuals (Lissner and Heitmann 1995; Heitmann *et al.* 1995; Ramachandrappa and Farooqi 2011; Xia and Grant 2013; Waalen 2014). The heritability of obesity in humans (as measured with BMI or body mass index) is between 40 and 70% (Barsh *et al.* 2000; Xia and Grant 2013), indicating that both genetics and environment have large influences on this trait. An individualized approach where the heritability of obesity and environmental factors for that individual are both considered is necessary for improving possible treatments. Understanding the genetic and environmental circumstances responsible for nutrient allocation to storage is vital to increasing our knowledge about obesity and metabolic syndrome (metabolic syndrome can be defined as a series of disorders including obesity, hypertension, insulin resistance, and various other cardiovascular risk factors that occur together and indicate an increased chance for cardiovascular disease (Huang 2009)). When we understand the genetic and environmental influences behind obesity, it affords us the opportunity to increase human health by being able to offer treatment recommendations specific to the individual.

To examine the genetic and environmental influences responsible for nutrient allocation, we used *D. melanogaster* as a model organism. Fruit flies are an excellent model organism for this study because we know that diet accounts 1 to 2% of variation in

their metabolic traits, genetics can account for between 11 and 23% variation in metabolic traits, and the interaction between genotype and diet can account for 12 to 17% variation (Reed *et al.* 2010b). Additionally, because 70% of the disease causing genes in humans can also be found in *Drosophila*, we are able to imitate insulin resistance and other metabolic syndrome factors (Musselman *et al.* 2011; Ugur *et al.* 2016; Dobson *et al.* 2017; Musselman and Kühnlein 2018). However, as with all model organisms, they do not perfectly align with humans: we use a model organism here as a tool.

Flies vary how and where they store they store nutrients. Some macromolecule content is stored for long term use, e.g. glycogen stores as a benefit to increased starvation resistance (Graves *et al.* 1992). Flies that have higher stores of lipid are correlated with higher starvation resistance (Clark *et al.* 1990). Glycogen is used as a resource during starvation and the *Drosophila* fat body accumulates several nutrients for storage, mainly in the form of triglycerides and glycogen. (Arrese *et al.* 2001; Arrese and Soulages 2010).Our previous paper indicated the macromolecule content had evolutionary potential, we are hoping to understand the long term effects of selection pressure on our base population (Clark 1990).

Organisms store nutrients differently according to nutrient environments (Williams et al 2015). While an organism on a high sugar diet may store more nutrients in the form of lipid or glycogen, after an organism is on a high sugar diet for several generations, they may no longer store nutrients in the same pattern. Plastic phenotypes exist when a single genotype responds differently in different environments (Fox *et al.* 2019). Plasticity in nutrient allocation is a useful strategy for dealing with changing nutrient environments (Sgro and Hoffmann 2004; Ng'oma Enoch *et al.* 2017).

Phenotypic plasticity will evolve as these traits are exposed to a particular selection regime over time. Phenotypic plasticity will be favored when flies are on a fluctuating or deteriorating nutrient availability(Hoffmann and Merila 1999). Over allocation to storage on the constant high nutrient availability diet will be selected against. Organisms may evolve different patterns of plasticity when resources are low in predictable environments or when exposed to a wider range of environments (Noach *et al.* 1996; Price 2006; King and Roff 2010).

We selected flies for 30 generations on three different nutrient treatment regimens: a deteriorating availability of nutrients, a fluctuating availability of nutrients, and a constant high availability of nutrients. At generation 30, we took the selection lines and placed each set of flies on one of three different food treatments and after ten days on this food we measured proteins, lipids, soluble carbohydrates and glycogen contents. Prior to measuring macromolecule content using an energy budget assay, we dissected the ovaries out of adult female fruit flies so we could measure macromolecule content in both somatic tissue and reproductive tissue.

Methods

Fly Husbandry and Experimental Population

We began experimental evolution using an admixed population from the *Drosophila* Synthetic Population Resource (DSPR). The DSPR is a multiparent mapping population, consisting of an A and B population. Two sets of fully sequenced founder lines (A1-A7 and B1-B7 plus a common founder line for both, AB8) were interbred for 50 generations. The ensuing Recombinant Inbred Lines (or RILs) were next inbred for 25 generations to produce a total of over 1500 Recombinant Inbred Lines or RILs between the two populations (flyrils.org; (King *et al.* 2012a; b). More information about the DSPR, including its generation, properties and statistics can be found at King et al (2012 a,b) and at http://FlyRILS.org.

The selection experiment crossing was done as a collaboration within the King lab. The original cross and 30 generations of maintenance were done by myself, Enoch Ng'oma(King lab postdoc) and various King lab technicians. I did not collect information on egg number or size as it was beyond the scope of this experiment, however, Enoch Ng'oma did, while measuring other life history traits. Sequencing was done by King Lab technicians.

Prior to the start of the selection experiment, we generated a synthetic population from the DSPR which served as our base population. Briefly, we took 5 randomly chosen females per RIL from 835 B population RILs of the DSPR and placed them in 6 cages with maintenance diet food. We collected eggs and placed them in glass milk bottles. Adult flies from the milk bottles were released into 6 cages and allowed to mate for 5 generations. Food dishes were changed 3 times weekly. For the following

generations, we collected eggs and transferred them to 30 vials per cage. Each vial had approximately 50-90 eggs. These vials of eggs were dispersed across the 6 cages to produce a genetically homogenous population. The five generations were on 3week cycles (oviposition to egg collection). We phenotyped the base population and estimated heritability by performing a half-sibling experiment before the start of the selection experiment.

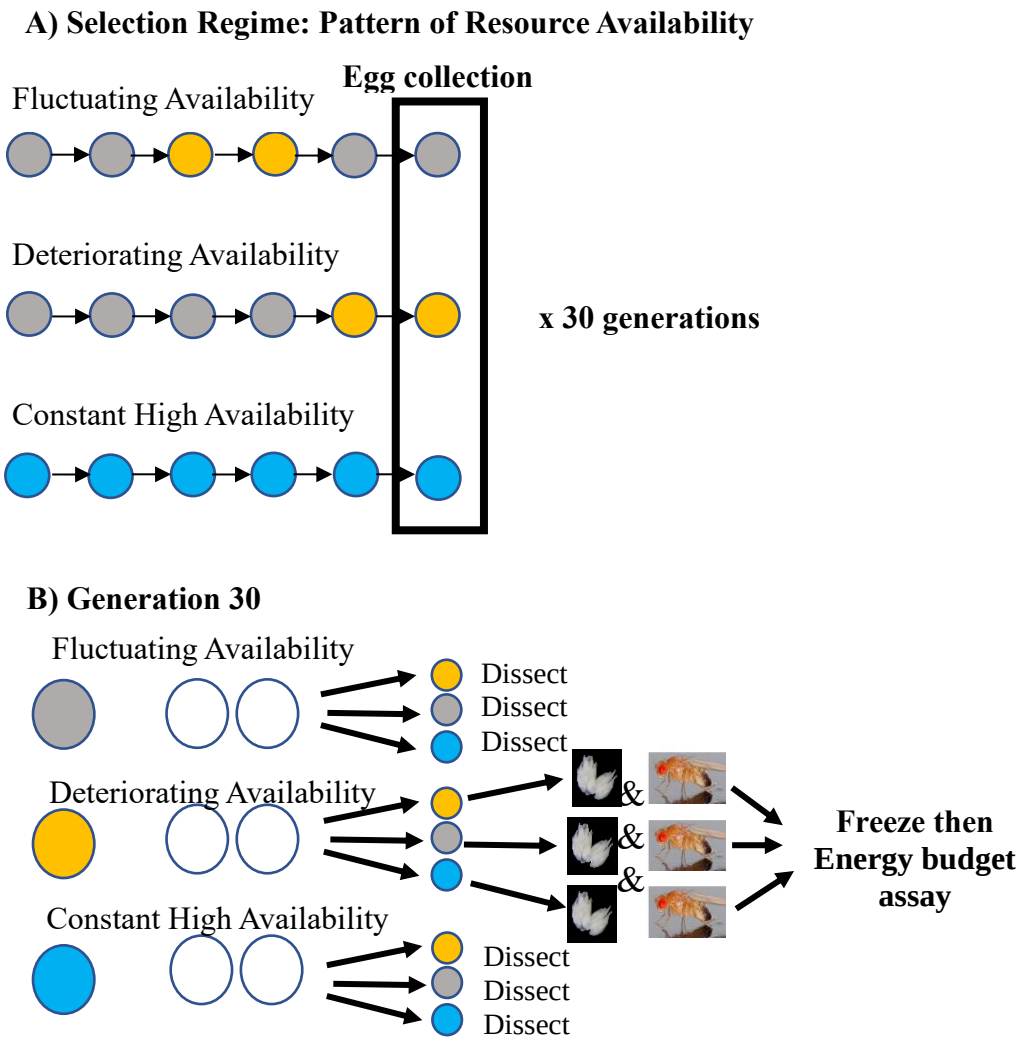
We maintained 36 selection lines in large cages on three dietary treatments with varying nutrient availability. We used the following treatments: fluctuating availability (FA), deteriorating availability (DA) and constant high (CH) availability of nutrients. We reared eggs until adulthood on maintenance food. On day 10, when the flies reached the adult stage, they were placed on these selection regimes. For the FA treatment, for the first 10 days the flies are on maintenance food (larval stage), days 10 to 14 they are on Control (C) food, Dietary Restriction (DR) food from day 14 to 18, and C food from day 18 to 21. For the DA treatment, for the first 18 days of adulthood, flies were on C food. Then, flies were on DR food from day 18 to 21. For the CH availability treatment, larvae were on maintenance food from day 1 to 10 until reaching adulthood; adults were on high sugar (HS) from day 10 to 21. These diets (C, DR, and HS) reflect environments of differing nutrient availability: HS reflecting high nutrient availability, and DR reflecting low nutrient availability. The differing nutrient availability was produced by altering amounts of sucrose and yeast in the DR and HS food treatment recipes. The DR diet has half of the yeast the C and HS diet. The HS diet contains nearly seven times the amount of sucrose compared to the C and DR diets. These diets were adapted from Bass (Bass *et al.* 2007) and Skorupa (Skorupa *et al.* 2008), chosen to ensure effects on lifespan and

fecundity. Reproduction is very reduced, relative to other diets and lifespan extended (Ng'oma *et al.* 2018).

At Generation 30, we phenotyped 12 selection lines, 4 from each of these selection treatments. Prior to any phenotyping, selection lines were placed on standard maintenance food for two generations to decrease the impact of environmental and parental effects from consideration. Following these two generations, we set up two vials of each diet (DR, C, HS) and line with 30 mated adult females, for a total of 180 flies per line. Flies from each vial were transferred to fresh food every two days for 10 days until flash frozen in liquid nitrogen and stored at -80C prior to dissection of ovarian tissue (all were dissected within one month of original freeze date) and remained in the -80C freezer until they were assayed.

Figure 1. Experimental Design Selection Experiment

Gray circles indicate C diet; orange circles indicate DR diet, and blue circles indicate HS diet.



Energy Budget Assays:

We modified Foray et al's ((Foray *et al.* 2012) energy budget assay for parasitic wasps to use on fruit flies. This is an assay that is itself a modified from Van Handel's original mosquito assay (Van Handel 1985; Van Handel and Day 1988) to run in a microplate. We measured proteins, lipids, and carbohydrates in dissected female fruit flies: ovaries and somatic tissue separately. We homogenized the ovaries/ somatic tissue of 10 female *D. melanogaster* with a stainless-steel bed and aqueous lysis buffer solution using a BeadBeater in an Eppendorf tube. Next, we aliquoted 2.5 microliters of the supernatant of this solution to measure proteins in the Bradford assay and used Bovine Serum Albumin (BSA) as the standard curve. We added chloroform-methanol and sodium sulfate to the supernatant, centrifuged and proceeded to measure carbohydrates and lipids from the resulting supernatant and glycogen from the pellet in borosilicate glass microplates. We used plastic microplates for the protein assay, as no volatile chemicals were used during the assay. We used borosilicate plates to prevent plastic contamination in the supernatant.

We measured carbohydrates by evaporating 150 microliters of supernatant in a borosilicate microplate to 10 microliters in a vent hood. Following a recommendation from Cheng et al, we increased the length of evaporation time in the vent to 40 minutes, instead of Foray's 15 minutes (Cheng *et al.* 2011; Foray *et al.* 2012). We incubated samples for 15 minutes at room temperature with anthrone reagent, and then incubated in a 90 degree water bath for 15 minutes. Once cooled, we measured the absorbance in a plate reader. Anthrone was also used to measure glycogen. We used dextrose in methanol as the standard curve for both soluble carbohydrates and glycogen.

We measured lipids by pipetting 100 microliters of supernatant and evaporating in a 90 degree water bath until dry. We then added vanillin and sulfuric acid to each well and used a chloroform standard curve to measure absorbance.

We used standard curves (a known concentration and total macromolecule amount) to convert sample absorbance to concentration then to amount. We used standard curves on each microplate for each macromolecule. We measured absorbances per well. Because each well was a specific proportion of the total solution, we calculated total macromolecule amount per well, per total solution, and further calculated per fly. Here, we present macromolecule amount in micrograms per fly. Every sample had at least one technical replicate, run concurrently within plate. For statistical analysis, we used the mean of these two samples.

Statistical Analysis

All analysis described below were performed in R (R Core Team 2018).

Pre-processing

We took individual plate output from the plate reader and compiled it into a single dataset. We found the mean for technical replicates within plates for each sample. From here, we produced two datasets; one, a "whole body" dataset where the ovarian tissue and somatic tissue for each sample was summed with each other to yield the macromolecule content per fly, and a second "dissected tissue" dataset, in which ovarian tissue and somatic tissue remained separate per sample. Repeatability measurements can be seen in Supplementary (Table S1). We identified outliers for each macromolecule as any value three standard deviations from the mean using the "whole body" dataset and removed

outliers from both datasets. When outliers were removed from the "dissected tissue" dataset, both the ovarian and somatic tissue samples were removed for that macromolecule. Proteins, carbohydrates, and glycogen each had 1 outlier that was removed. There were no lipid values that fell under the 3 standard deviations away from the mean criteria.

Amounts acquired plots:

We produced plots, measuring the amount of macromolecules across three diets, or amounts acquired plots, for each macromolecule using untransformed, absolute values for both the summed whole bodies as well as separate plots for ovarian tissue and plots for somatic tissue. We plotted the mean of each line across diet treatments. For the second set of plots, we plotted the mean of diet treatment across selection lines.

Transformations

Next, to check for normally distributed data, we produced histograms for each macromolecule across each diet. We transformed macromolecule contents to a normal distribution if they did not already adhere to a normal distribution. The protein, carbohydrate and lipid samples remained untransformed. Glycogen on HS and C foods were untransformed; however, for glycogen on the DR diet, we took the square root of glycogen on DR to adhere to a normal distribution. We transformed data until a normal distribution in order to run statistical tests that required a normal distribution of data.

ANOVA

We conducted an Analysis of Variance (ANOVA) to determine the effect of diet on macromolecule amount, using data adhering to a normal distribution. We fit a linear model for individual macromolecule content as modeled by diet and Linetype. We used

the ‘lme’ function in R from the ‘nlme’ package (Pinheiro et al) to fit the following linear model:

*(macromolecule ~ LineType * diet, random=~1|Line)*

Where LineType (DA, FA, CH) was a fixed effect and Line (selection line: 1.DA, 2.FA etc) was a random effect. An effect from Linetype * diet would indicate different effects of diet in selection treatment, or Linetype diet interaction We then used ‘anova.lme’ to measure the effect of treatment on macromolecule. Our dataset was missing the following values: 1.DA.C and 1.DA.HS for all macromolecules; 2.DA.HS for carbohydrate; 3.DA.C for protein; and 2.FA.HS for glycogen. We followed up with Tukey tests to account for the multiple tests performed.

Results

Phenotypic Patterns

We dissected the ovaries of female fruit flies and measured protein, lipid, soluble carbohydrates and glycogen amount in ovaries and somatic tissue across 3 different diets. We fit a linear model using transformed amounts per fly modeled by diet for each macromolecule. We performed a mixed model ANOVA (Analysis of Variance) on each macromolecule and performed post-hoc testing in the form of Tukey tests.

First, we observed protein amount measurements (Figure 2, A-C). Highest protein acquisition on the C food was seen across the whole body, somatic and ovarian tissue. Somatic tissue protein acquisition showed more variation. Thus, so did whole body protein acquisition. Ovarian tissue had the highest protein acquisition on the C food and lowest on HS food and showed less variation.

Whole body lipid acquisition was lowest on the C food and highest on the HS food. This pattern was strongly mimicked in lipid acquisition in somatic tissue. Oddly, in ovarian tissue, the highest lipid acquisition was on C food, followed by HS, then DR foods (Figure 2, D-F). For the selection flies, highest and lowest lipid acquisition by diet differed by tissue. In contrast to the flies measured in the base population (Chapter 3), the smallest amount of lipid acquisition occurred on C diet and highest on HS diet.

Next, we looked at the whole body carbohydrates (Figure 2, G-I) amount measurements. In the summed whole body, we saw low amounts of carbohydrate on both the DR and C foods and significantly more carbohydrate acquired on HS food (a highly similar pattern see in carbohydrate acquisition measured in the base population: See Chapter 3). Not unsurprisingly, carbohydrate acquisition in somatic tissue closely

mimicked this pattern. This is largely in part because the amount of nutrients stored in ovarian tissue is but a small fraction of the whole body. Carbohydrate acquisition in ovarian tissue showed significantly more variation, where DR, C and HS means scale up as calories scale up. Beyond this, there were no strong pattern of carbohydrate acquisition discernible in ovarian tissue.

Lastly, we looked at overall glycogen content (Figure 2, J-L). Glycogen, an insoluble form of carbohydrate, showed tremendous variation between acquisition on DR, C and HS foods. Unlike every other macromolecule listed here, we observed comparable amounts of glycogen acquisition between somatic tissue AND ovarian tissue. Overall, glycogen whole body and somatic tissue acquisition showed similar patterns: where the lowest amount of glycogen was acquired on DR and the highest on HS. Ovarian tissue shows the opposite pattern, where the highest amount of glycogen was acquired on DR whereas the lowest amount of ovarian glycogen was acquired on the HS diet. In contrast, in the base population (Chapter 3), the smallest amount of glycogen acquisition occurred on the C diet and highest on DR and HS. Tukey tests indicated acquired glycogen within the whole body was significant when C and HS were compared to DR. There was a great deal of variation due to diet for both soma, ovaries and thus, the whole body.

The results of the mixed model ANOVA indicate on every macromolecule non-significant linetype: insignificant effect of selection treatment. However, there was a significant effect of diet for carbohydrate, lipid and glycogen. There was no significant linetype-diet interaction. Had there been significant linetype-diet interaction, it would have suggested the evolution of different patterns of plasticity.

However, we did notice trends: carbohydrate had similar patterns of acquisition between the base population and the selected lines. Basic acquisition between DR and C diets was less than 0.05 micrograms for both. However, the base population had nearly double the amount of carbohydrate acquired on the high sugar diet. Patterns of protein acquisition were similar between the base population and generation 30 of the selection experiment. However, the selection lines had overall a higher range of protein acquisition, acquiring up to 0.05 micrograms versus the half-sibling experiment's 0.04 micrograms of protein. Selection lines acquired overall less lipid amounts than the base population, though the pattern of acquisition of the C diet acquiring the least amount of lipids and the HS acquiring the most amount of lipids remained similar. Lastly, glycogen acquisition in the selection lines was far more variable than the base population and on average the selection lines acquired less glycogen than the base population. However, there were two half sibling families that acquired twice as much glycogen on the HS diet than any line in the selection experiment.

We also plotted macromolecule amounts across diet treatments (Figure 3), CH, DA and FA where the colored lines denote food treatments (DR, C, HS) rather than selection lines. For carbohydrates, we can extrapolate the same information from the previous set of plots: that HS food acquired more carbohydrates across every selection line, though less was acquired on the DA diet treatment. Glycogen again showed a great deal of variation across selection diet treatments, though. While the highest amount of glycogen was acquired on selection lines experiencing CH availability of nutrients, the least amount of glycogen was acquired on selection lines experiencing FA treatment. Lipid showed that HS lines notably acquired more lipid across all treatments, but that

least amount of lipid was acquired on the DA selection treatment and highest, unsurprisingly, on lines that underwent the CH selection treatment. Finally, protein showed the same pattern of most protein acquired on lines that underwent CH selection treatment and least on DA selection treatment but even stronger, but lines that finished on HS did not show high protein acquisition over other food treatments.

Table 1. Means and SE of Amounts acquired plots

	DR	DR	C	C	HS	HS
	Mean	SE	Mean	SE	Mean	SE
Carbohydrate (whole)	.0320	.00245	.03195	.001715	.07149	.00556
Ovaries	.0109	.000786	.01228	.000942	.0131	.000826
Soma	.0243	.001199	.0195	.000669	.06265	.00377
Glycogen (whole)	.402	.0358	.4446	.0277	.4456	.0366
Ovaries	.249	.0265	.237	.0187	.221	.01995
Soma	.188	.0122	.201	.0142	.2478	.0194
Lipid (whole)	7.112	.427	6.888	.2894	8.724	.518
Ovaries	2.321	.101	2.682	.102	2.663	.0919
Soma	5.422	.189	4.298	.144	6.820	.2976
Protein (whole)	.00336	.000217	.00399	.000136	.00347	.000184
Ovaries	.000839	5.021e-5	.00101	5.64e-5	.000789	3.98e-5
Soma	.00291	6.36e-5	.0031	7.225e-5	.00295	6.704e-5

Table 2. Analysis of variance for macromolecule amount

Source refers to macromolecule, df is the abbreviations for degrees of freedom, MS stands for Mean Square, P indicates significance for protein, lipid, glycogen and carbohydrates.

Carbohydrate	**numDF**	**DenDF**	**F value**	**P value**
Intercept	1	15	811.7354	<.0001
Linetype	2	9	1.1765	.3516
diet	2	15	79.2398	<.0001
Linetype: diet	4	15	.1203	.9731

Lipid	**numDF**	**DenDF**	**F value**	**P value**
Intercept	1	16	1251.8398	<.0001
Linetype	2	9	0.4294	.6635
diet	2	16	16.8981	.0001
Linetype: diet	4	16	0.4188	.7927

Protein	**numDF**	**DenDF**	**F value**	**P value**
Intercept	1	15	1373.1271	<.0001
Linetype	2	9	.6599	.5402
diet	2	15	3.4315	.0593
Linetype: diet	4	15	1.3333	.3030

Glycogen	**numDF**	**DenDF**	**F value**	**P value**
Intercept	1	15	644.3661	<.0001
Linetype	2	9	.6439	.5478
diet	2	15	60.0391	<.0001
Linetype: diet	4	15	1.7829	.1848

Figure 2. Amounts Acquired Plots by Diet

Macromolecule is plotted across diet. Color indicates selection treatment lines: Purple is Deteriorating Availability of Nutrients, Green is Constant High Availability of nutrients, Pink is Fluctuating Availability of Nutrients. The square points indicate treatment averages on each diet.

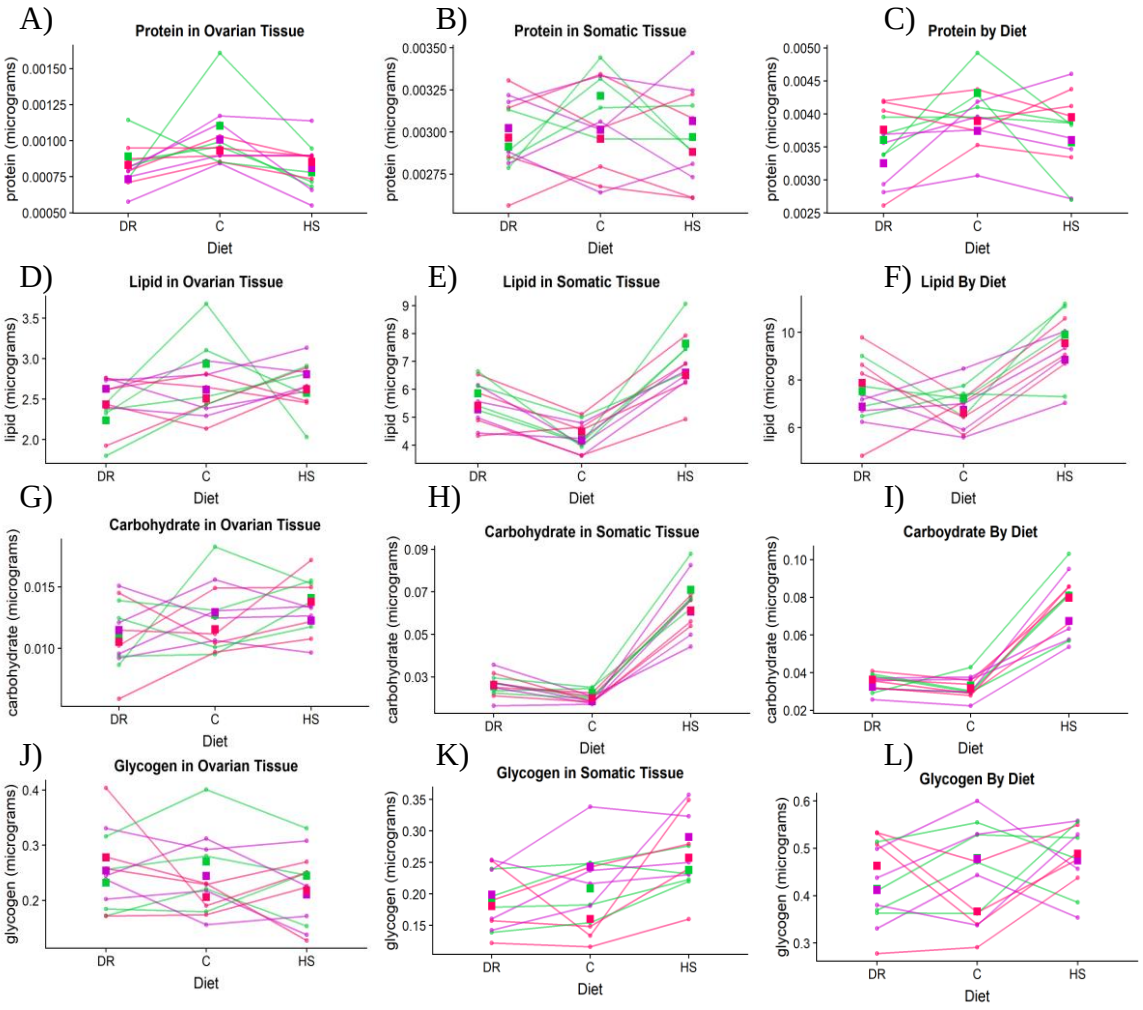
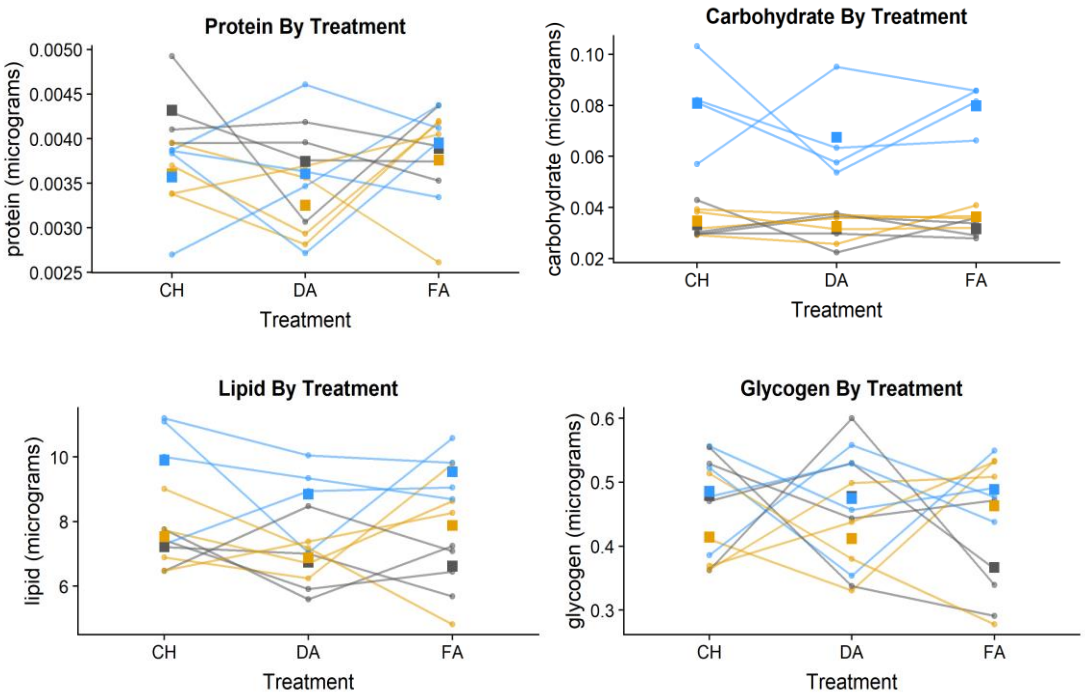


Figure 3. Amounts Acquired Plots by Treatment

Macromolecule is plotted across selection treatment. Color indicates diet: Blue is HS, Orange is DR, Grey is C. Square points indicate diet means on each selection treatment.



Discussion

In this study, we performed a selection experiment to measure the impact of three different selection regimes on the overall body composition of female fruit flies as well as allocation of nutrients to reproductive tissue versus somatic tissue. For 30 generations, we put flies on one of three selection regimes: constant high availability of nutrients (CH), deteriorating availability of nutrients (DA) or fluctuating availability of nutrients (FA). Prior to phenotyping, we put each selection line on one of three diets so we could test if there was an interaction between diet and selection treatment. While there have been selection experiments to examine the effects of selection regimens on different traits in *D. melanogaster* before (e.g. desiccation resistance, metabolic rate), far fewer have carried on selection in variable environments past 20 generations and measured total energy budget within *drosophila*. (Clark *et al.* 1990; Chippindale *et al.* 1993, 1997; Sgro and Hoffmann 2004; Baldal *et al.* 2006; Albers and Bradley 2006; Burke and Rose 2009)

Phenotypic Patterns

We used Foray's energy budget assay to measure macromolecule content within reproductive and somatic tissue in female *D. melanogaster* (Van Handel 1985; Foray *et al.* 2012 p. 200). Nutritional environment affects nutrient storage as well as nutrient acquisition (Sgro and Hoffmann 2004). We wish to see the effects of selection by using different diet treatments on adult *drosophila*; experiments have been performed altering the nutritional environment altering the nutritional environment of *drosophila* at the larval stage (Aguila *et al.* 2007b; Pascacio-Villafán *et al.* 2016). Prior to phenotyping

we put each line on maintenance food for two generations to eliminate parental and environmental effects (Dew-Budd, Jarnigan, and Reed 2016).

Acquisition

In terms of acquisition, most strikingly, between the base population and the selected lines, carbohydrate acquisition on the selection lines largely mimicked patterns of acquisition in the base population, though the selected lines did acquire less carbohydrate on the HS diet than the base population flies on HS. Protein acquisition in the selection lines was more variable than protein acquisition in the base population. The selection lines acquired less glycogen and lipids and were more variable than the base population.

Effects of Treatment

We expected significant treatment effects. The results of the ANOVA indicate that there was no significant effect of line type. Neither was there line-diet interaction. One would expect that they respond to changes in diets in similar ways. We would expect, for example, that on the CH treatment, flies would lose plastic phenotypes. However, that does not appear to be the case. Specifically, when we look at carbohydrate acquisition between both populations, the striking similarity after thirty generations of selection in a nutrient environment is highly unexpected. Djawdan et al. ((Djawdan *et al.* 1998), for example, found higher levels of both lipid and carbohydrate on fly lines selected for starvation resistance. Our results do not confirm this. Harshman and Hoffman ((Harshman and Hoffmann 2000) indicated that selection experiments, even with similar selection pressures, produce inconsistent responses. And while effect of treatment was not significant, effect of diet was statistically significant. This being

said, there does appear to be sufficient indications that it would be promising to continue for more than 30 generations, if only to see if this pattern changes. Selection experiments continue to be valuable methods to understanding resource allocation.

One possible explanation for the lack of change in carbohydrates is that flies on the CH treatment engaged in compensatory eating (Carvalho *et al.* 2005; Tessnow *et al.* 2018), which may explain why carbohydrate acquisition and proportion were so similar between the selected lines and the base population. However, this does not explain the similarity between the DA and FA lines and the base population.

One interesting trend that we noted was that flies on the CH treatment acquired lipid at the same level as, and in some cases more lipid, than flies on the DA and FA treatments. One might expect that after thirty generations of being on the CH treatment, flies would have adapted and proceeded to store less lipid. That they did not indicates interesting questions concerning selection pressure and at what point a CH treatment will select for flies that store less lipid, or any macromolecule at the same rate.

Limitations

As with all model organisms, there are limitations— if flies were a perfect model for human metabolism and energy budget, we would not be using flies any longer. we are using flies as a simpler tool for a complex system. Beyond the basic flies are insects versus humans are mammals, there are other important factors differing between humans and *Drosophila*. First, humans can store nutrient sources outside the body, for example in pantries and fridges. Second, there is a wide range in the diets of humans, and they require different nutrients for different tasks. Humans do not fly or

lay eggs, for example. Further, the flies in this experiment are raised on lab food in a lab. Flies in the wild would feed on decaying fruits.

Conclusion

In the last several generations, the problem is no longer low nutrient resources environments or even fluctuating nutrient resources, but overabundance of nutrients and calories. It is no longer advantageous for humans to store the majority of nutrients and order for humans to adapt, there must be a fitness cost to storing nutrients in high resource environments. Given the results of our experiment, it appears that it will take more than 30 generations for us to acclimate to constant high availability of resources. Of course, for human, the picture is far more complex than internal nutrient storage. We know that humans at extra-somatic food storage and have been for generations. However, these findings, on the issue of response to nutrient selection, and plasticity of macromolecule content, provide an interesting first step in understanding the bigger picture of nutrient allocation and acquisition in humans.

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Supplemental

Table S1. Repeatability measurements

	Carbohydrate	Glycogen	Protein	Lipid
Repeatability	95	91	86	83

CHAPTER 5: Conclusion

This dissertation examined the genetic and phenotypic variation in macromolecule content, estimated heritability for these phenotypes, and studied the effects of selection on macromolecule content.

In Chapter 2, I measured the effect of nutritional environment on overall fly composition. By using the energy budget assays, I show that there is phenotypic variation in response to diet, the genotypes responsible for nutrient content storage are plastic and that there are multiple genomic loci of interest. Nutrient acquisition increased according to diet composition, with DR having the lowest amount and HS having the highest. The exception to this pattern was glycogen. On the C diet, lipid and carbohydrate amounts correlated together. Overall, protein consistently correlated with all other macromolecules between 0.2 and 0.3 correlation. Unlike previous studies, I focused on the impact of diet and measured all four energy budget components on the same individual flies. This allows a wider understanding of resource allocation in different environments.

In Chapter 3, I estimated the heritability of lipid, carbohydrate, glycogen, and protein contents across three different diets using a half-sibling design experiment. I showed differing heritability across nutritional environments for different macromolecule contents, suggesting not only does nutrient content change based on the particular environment for a genotype, but that these phenotypes are heritable.

In Chapter 4, I tested the effects of female fruit flies undergoing selection for 30 generations. I measured protein, lipid, soluble carbohydrates, and glycogen amount in

ovaries and somatic tissue across three different diets across three different selection regimes and found that selection treatments after 30 generations did not significantly impacted macromolecule content. However, diet did. Strikingly, for carbohydrates specifically, patterns of acquisition remained the same in both the base population and after thirty generations of selection regardless of selection regimen. It is possible that 30 generations was not the optimal length of evolutionary time to show a significant change in acquisition in response to selection.

In conclusion, there is variation in macromolecule content acquisition. It is a heritable phenotype. There are several loci responsible for macromolecule content acquisition.

Future Directions

In Chapter 2, the large number of genome scans limited our ability to have a meaningful significance threshold for QTL peaks, to the point where no QTL peaks reached significance. Given unlimited time, I would pursue QTL mapping of the RIX further, which would involve vastly larger sample sizes. With increased sample sizes (and increased accuracy from the optimizations) new QTL peaks may arise. I would like to study those further. Should any candidate genes arise from these new suggestive regions, I would like to see the effects of knockout studies. Second, I would dissect the ovarian tissue from RIX female flies and do genetic mapping with the energy budget assay results from somatic and ovarian tissue separately. While I dissected reproductive tissue from female fruit flies in both the half sibling and experimental evolution studies, neither approaches lend themselves to QTL mapping. With regards for Chapter 3, if the

heritability experiment were re-run, I would not dissect out the ovarian tissue again. Because of the nature of sample collection, these samples were not able to be used in the animal model. For these flies, I would also increase the length of time they were on the experimental food and place them on it earlier.

In terms of Chapter 4, I would like to examine the effects of raising larva on experimental foods rather than maintenance food prior to adulthood, to see whether the results we see in selection are magnified by these changes, or whether they go in the opposite direction due to a changed nutritional environment during the larval stage. The selection experiment would proceed the same way following this change in larval diet. Broadly, one unexplored effect is the GI microbiota of these flies. Lab raised flies have different GI microbiota than do wild flies. And while we try to limit microbial interference in the experimental and maintenance food made for the flies, it is still present and we do not know the full extent of microbial interaction across the different diets.

In a slightly different direction, research coming from the Reed lab at the University of Alabama has approached gene by environment interactions similarly to the King lab by testing flies in nutrient environments, but they also use flies in a high fat environment. However, they have not run long term selection experiments. One interesting avenue of study would be to run an experimental evolution study and include a high fat diet in addition to DR, C, and High Sugar diets. Likewise, I would also like to examine additional regimen's such as one with constant low availability of nutrients and put flies that had been on a constant high availability of nutrients for several generations on a new selection regimen. For example, I would take CH lines and put them on DA or FA selection treatments and examine the effect after ten generations. I would like to see

whether these changes in selection regimen would affect macromolecule content of future generations. Given more time, I would also continue the selection experiment into Generations 50, 75 and 100. Perhaps, after more generations, we would see greater differences in nutrient allocation.

Summary

Chapter 2: Genetic & Physiological Basis of Energy Budget in DSPR Flies

- Macromolecule content is a plastic phenotype
- We were not able to identify any significant QTL peaks for carbohydrate, lipid, and protein content.
- However, we were able to identify some suggestive regions of interest.
- There is variation in macromolecule content acquisition in response to diet.

Chapter 3: Pattern of Heredity of Carbohydrate, Lipid, and Protein Contents in Different Nutritional Environments

- We observed strong patterns of carbohydrate acquisition, with the least amount of carbohydrate acquired on the DR and C diets and dramatically more acquired on the HS diet. There were higher heritabilities on the HS and DR diets than the C diet.
- The was a great deal of variation in glycogen content acquisition within diets, with the least amount of glycogen acquired on the C diet. There was moderate heritability of glycogen content across diets.
- The highest protein acquisition was on the Control diet and the least on the DR diet. There was high protein heritability across diets.

- Lipid acquisition was lowest on the C diet and highest on the HS diet.
- There is differing heritability for different macromolecule contents across nutritional environments.
- Nutrient content changes based on the particular nutritional environment the genotype is in.

Chapter 4: Effects of Diet-Based Selection on Macromolecule Content in Drosophila Melanogaster

- Diet is more influential than selection regime: Effect of treatment was not statistically significant, whereas, effect of diet was statistically significant.
- Between the base population and the selected lines, carbohydrate acquisition were similar
- Protein acquisition in the selection lines was more variable than protein acquisition in the base population.
- Highest amount of glycogen acquisition on diets in the selection lines differed by tissue: in ovarian tissue it was the DR diet, in somatic tissue it was the HS diet. Similar amounts of glycogen were acquired in both the somatic and reproductive tissues.
- Lipid acquisition followed a similar pattern to glycogen acquisition, however considerable more lipid was acquired in the somatic tissue than reproductive tissue.

VITA

Anna Perinchery-Herman was born in Chicago, Illinois on 1 December 1991 to Princy and Vinny Perinchery. She attended school in Illinois and Kansas and graduated in 2010 from Blue Valley High School in Stilwell, Kansas. In 2014, she earned a BS in biology from Newman University in Wichita, Kansas. While in Wichita, she was employed as a biology and chemistry tutor as well as a Lab Assistant and Teaching Assistant for the following courses: General Chemistry 1 and 2 and Cell and Molecular Biology; and as a Teaching Assistant for Human Anatomy and General Genetics. In Fall 2014, Anna moved to Columbia and began her doctoral studies under Dr. Elizabeth King at the University of Missouri. She defended her dissertation in April 2020. Anna currently resides in Arkansas with her husband, Stephen Perinchery-Herman and dog, Bella.