The Role of Developmental and Adult Diets in Modulating Reproductive Senescence: Insights from *Drosophila melanogaster*

A Thesis

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by

Ruchitha Byrohalli Gangaiah

Indian Institute of Science Education and Research Pune

Dr. Homi Bhabha Road,

Pashan, Pune 411008, INDIA.

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Under the guidance of

Supervisor: Dr. Sudipta Tung,

Ashoka University

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INDIAN INSTITUTE OF SCIENCE EDUCATION AND RESEARCH PUNE

Certificate

This is to certify that this dissertation entitled "The Role of Developmental and Adult Diets in Modulating Reproductive Senescence: Insights from *Drosophila melanogaster*" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Ruchitha Byrohalli Gangaiah at Ashoka University under the supervision of Dr. Sudipta Tung, Wellcome Trust-DBT India Alliance Early Career Fellow, Department of Biology, during the academic year 2023-2024.

Sudipta Tung

Dr. Sudipta Tung

Committee:

Dr. Sudipta Tung

Professor Sutirth Dey

This thesis is dedicated to Nico and Debosatya

Declaration

I hereby declare that the matter embodied in the report entitled "The Role of Developmental and Adult Diets in Modulating Reproductive Senescence: Insights from *Drosophila melanogaster*" are the results of the work carried out by me at the Department of Biology, Ashoka University, under the supervision of Dr. Sudipta Tung and the same has not been submitted elsewhere for any other degree. Wherever others contribute, every effort is made to indicate this clearly, with due reference to the literature and acknowledgement of collaborative research and discussions.

Ruchitha Byrohalli Gangaiah

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Abstract

Several studies have reported greater reproductive output in populations reared in rich developmental diets; while, several other studies have reported lower reproductive ability in populations reared in rich developmental diets, as compared to populations reared in poor developmental diets. I attempt to resolve this ongoing debate by highlighting the age-dependent effect of developmental diet on reproductive output. My results clearly demonstrate that the populations reared in rich developmental diet have greater reproductive output in early-life, but lesser reproductive output in mid-life as compared to populations reared in poor developmental diet. I also find that there is a gradual shift from reproductive output being affected only by developmental diet in very-early-life to reproductive output being affected by both developmental and adult diets in early-life and mid-life to reproductive output being affected only by adult diet in late-life. I also find that although the number of viable progeny produced is dependent on the effect of both developmental and adult diets across age, the various physiological processes involved in egg production are not necessarily dependent on both the diets. Specifically, the number of ovarioles are dependent only on developmental diet; while, ovary size and the proportion of egg chambers that undergo vitellogenesis are dependent on both developmental and adult diets, and vary with age. These results address some of the key conflicts that exist in literature about the effect of developmental diets on adult reproductive output and highlight the importance of measuring adult traits in an age-specific manner.

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Contributions

Contributor name	Contributor role
Ruchitha BG, Sudipta Tung	Conceptualization Ideas
Ruchitha BG, Sudipta Tung	Methodology
Ruchitha BG	Software
–	Validation
Ruchitha BG, Sudipta Tung	Formal analysis
Ruchitha BG, Devashish Kumar, Itibaw Farooq, Nishant Kumar, Chand Sura, Chandrakanth M, Chetan S, Sudipta Tung	Investigation
Sudipta Tung	Resources
Ruchitha BG	Data Curation
Ruchitha BG	Writing - original draft preparation
Ruchitha BG, Sudipta Tung	Writing - review and editing
Ruchitha BG	Visualization
Sudipta Tung	Supervision
Sudipta Tung	Project administration
Sudipta Tung	Funding acquisition

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[1] https://journals.biologists.com/jcs/pages/author-contributions

Chapter 1 Introduction

Environmental conditions during development influence the expression of traits in adult life. Particularly, the diet consumed during development impacts the individual's health throughout their life (Black *et al.*, 2013). Thus, several attempts have been made to understand the effect of developmental diet on adult traits. In 1988, based on inferences drawn from several fertility studies where early food availability was documented (Sadler, 1969), Grafen proposed the silver spoon hypothesis. The silver spoon hypothesis posits that organisms reared on a better quality developmental diet have better fitness traits as adults (Grafen, 1988). Soon after, in 1992, Hales and Barker proposed the thrifty phenotype hypothesis to explain the occurrence of type 2 diabetes in humans as a mismatch of diets during developmental and adult stages of life. The thrifty phenotype hypothesis states that individuals who experience poor nutrition during developmental stages, program their metabolism with the expectation of poor nutrition in adult life. And so, if the quality of nutrition improves during adult life, the individuals' adaptation to poor nutrition now becomes maladaptive and they suffer from metabolic disorders like type 2 diabetes (Hales and Barker, 1992). Following this, in 2004, Gluckman and Hanson proposed a more generalized version of the thrifty phenotype hypothesis called the predictive adaptive responses (PAR). PAR states that irrespective of the shift in developmental diet to adult diet being poor to rich or rich to poor, the individuals will suffer a fitness cost in adult life due to maladaptation to the change in adult diet as compared to their developmental diet (Gluckman and Hanson, 2004). Since the inception of these hypotheses, various studies have been published in support of the silver spoon as well as the thrifty phenotype and the PAR hypotheses (Madsen and Shine, 2000; Van De Pol *et al.*, 2006; Millon *et al.*, 2011; Wong and Kölliker, 2014; Pigeon *et al.*, 2019; Duxbury and Chapman, 2020). However, particularly in recent years, there have been reports of instances in which the observed effects of developmental environment conditions on adult traits cannot be explained by the silver spoon or the thrifty phenotype/PAR hypotheses. Populations reared in poor developmental diets (and rich adult diets) have been seen to have greater reproductive output as compared to populations reared in rich developmental diets, which is incompatible with the predictions from any of the above hypotheses (May *et al.*, 2015; Spagopoulou *et al.*, 2020).

Within the past decade, this has led to several studies that attempt to explain the above-stated disagreement in the effect of developmental diet on adult traits by highlighting the context dependencies of the above hypotheses. In one such study, in 2015, May et al. empirically show that the effect of developmental diet on adult traits varies depending on adult diet and mating status. They demonstrate that when the adult diet is supplemented with yeast (source of protein) and they have continuous access to mates, populations reared on a rich developmental diet have decreased adult reproductive output as compared to populations reared on a poor developmental diet i.e. the silver spoon and the thrifty phenotype/PAR hypothesis cannot explain the effect of developmental diet on adult reproductive output when the adult reproductive potential is not constricted by unavailability of mates or dietary proteins (May *et al.*, 2015). Further, in 2020, Duxbury and Chapman find that the effect of developmental diet on reproductive output is seen only in females, and not in males. Even within females, they see that the silver spoon and thrifty phenotype/PAR hypotheses hold true when adult diet is high in protein; and only silver spoon hypothesis holds true when adult diet is low in protein (Duxbury and Chapman, 2020). Adding to this body of knowledge, in this thesis, I have attempted to further explore the nuances in how developmental and adult diets affect reproductive output in adult life.

Reproductive ability varies with age (May *et al.*, 2015; Duxbury and Chapman, 2020). Thus the effect of developmental and adult diets on reproductive ability of an organism would also vary with age. Additionally, reproduction is affected by various aspects of the diet like calorific value, macronutrient composition, etc. (Lee *et al.*, 2008). However, studies that look at the effect of developmental and adult diets on reproductive output change both calorific value and macronutrient composition of the diet simultaneously (May *et al.*, 2015; Duxbury and Chapman, 2020). Therefore, one cannot

distinguish between the effects of the two. In my study, I use *Drosophila melanogaster* populations reared in isocaloric dietary combinations of varying macronutrient compositions provided at developmental and adult life stages to study how reproductive ability varies with age. Using isocaloric diets removes the confounding effect of the calorific value to the diets that vary in macronutrient composition. I only vary the two major macronutrients – proteins and carbohydrates, and use a full-factorial combination of the diets applied to developmental vs. adult life stages. I use this system to examine the effects of the developmental and adult dietary combinations on the number of viable progeny produced in early, mid and late adult life. I observe the silver spoon theory is true in early-life (i.e. high protein developmental diet results in more viable progeny, irrespective of the adult diet); while, neither silver spoon nor the thrifty phenotype/PAR hypotheses are applicable in mid-life (i.e. low protein developmental diet results in more viable progeny, irrespective of the adult diet); and, the developmental diet does not affect the number of progeny produced in late-life. To further understand the underlying physiological basis of these differences, I examine how these dietary combinations affect the process of egg production since ovary formation and oogenesis regulation are known to be affected by inputs from nutrient-sensing pathways (Bownes, 1989; Green and Extavour, 2014).

Overall, the results from my study highlight the age-dependent nature of the effect of developmental and adult diets on adult reproductive output. Additionally, by examining the underlying physiological processes involved in reproduction in *D. melanogaster* females, I also show that although the number of progeny produced is dependent on the effect of both developmental and adult diets across age, the various physiological processes involved in egg production are not necessarily dependent on both the diets. I find that the effect of developmental diet on fecundity is regulated via ovarioles formation and vitellogenesis; while, the effect of adult diet on fecundity is mostly regulated only via vitellogenesis. These results address some of the key conflicts that exist in literature about the effect of developmental diets on adult reproductive output and highlight the importance of measuring adult traits in an age-specific manner.

Chapter 2 Materials and Methods

2.1. Experimental population

We used a large (n = ~2400) outbred population of *Drosophila melanogaster* maintained on a corn-media diet of protein to carbohydrate ratio of 0.4 for at least 35 generations. Progeny of this population were subjected to four experimental isocaloric diet regimes with varying protein to carbohydrate ratios across developmental and adult stages – HH, HL, LH, LL. (The first letter indicates the developmental diet and the second letter indicates the adult diet; where H represents high protein diet and L represents low protein diet). Experimental flies were reared on an uncrowded density of ~150 eggs per 50 mL of developmental diet. All assays were performed on flies from these experimental regimes by sampling the population cross-sectionally.

2.2. Experimental diet

The ancestral diet is a corn-media with a protein to carbohydrate ratio of 0.4. For a high protein diet, we used a protein to carbohydrate ratio of 0.7; and for a low protein diet, we used protein to carbohydrate ratio of 0.25 (Mudunuri *et al.*, 2024). All the diets used are isocaloric in nature. Detailed information on the diets can be found in Mudunuri *et al.*, 2024.

The ancestral population was raised on a diet of protein to carbohydrate ratio of 0.4 in both developmental and adult stages. The experimental flies were raised on the following diets to form a full-factorial combination of developmental and adult diets– high protein diet in developmental and adult stages (HH), high protein diet in developmental stage followed by low protein diet in adult stage (HL), low protein diet in developmental and adult stages (LL), and low protein diet in developmental stage followed by high protein diet in adult stage (LH). In the notation used, the first letter represents the developmental stage diet and the second letter represents the adult stage diet; H stands for high protein and L stands for low protein.

2.3. Assays

2.3.1. Viable progeny count

Two males and two females were aspirated into each glass vial, containing about 5mL adult food for 24 hours. There were 30 such vial replicates per treatment. After the 24-hour egg-laying window, replicates with dead females were discarded. The remaining vials were then incubated for 13 days to give sufficient time for the eggs to develop into adults. The eclosed adults were counted to quantify the number of viable progenies.

2.3.2. Ovary size measurement

Ovaries of 30 females from each treatment were dissected in 1X Phosphate-buffered saline (PBS). The images of the dissected ovaries were captured immediately under a stereomicroscope to determine the size of the ovaries. To quantify the size of the ovary, Region of Interest (ROI) was drawn around the ovary and area of the ROI was measured in ImageJ2.

2.3.3. Ovariole count

Ovaries of 30 females per treatment were dissected in 1X Phosphate-buffered saline (PBS). The dissected ovaries were placed in 4% Paraformaldehyde solution for 15 minutes to reduce the chances of breakage of ovarioles during further dissection. The ovaries were then shifted back to 1X PBS, and

the ovarioles of each ovary were separated and counted under light microscope to quantify the number of ovarioles per female.

2.3.4. Egg chambers maturation quantification

Ovaries of 20 females per treatment were dissected in 1X Phosphate-buffered saline (PBS). The dissected ovaries were placed in 4% Paraformaldehyde (PFA) solution for 15 minutes. The ovaries were then shifted back to 1X PBS, and the ovarioles separated. Egg chambers in pre-vitellogenesis and that undergo vitellogenesis were counted under light microscope. Oocyte being larger than the largest nurse cell was taken as the marker for vitellogenesis (Bakken, 1973). So, all egg chambers with oocyte smaller than the largest nurse cell were binned as pre-vitellogenesis egg chambers; whereas, all egg chambers with oocyte larger than the largest nurse cell were binned as egg chambers that undergo vitellogenesis.

2.4. Experiments

2.4.1. To investigate the effect of developmental and adult diets on age-dependent reproductive output

To understand how developmental and adult nutrition interact across age to influence reproductive output, we counted the number of viable progeny produced by flies reared in HH, HL, LH and LL diets in early, mid and late stages of adult life i.e. on days 10, 12, 22 and 32 from the day of egg collection. In total, we counted viable progeny produced by 480 male-female pairs for this experiment – 30 male-female pairs x 4 dietary regimes x 4 age-points.

The last age point i.e. day 32 was chosen as the day before the start of demographic senescence in the population (Mudunuri *et al.*, 2024) to avoid survivorship bias in the data. Day 10 represents the day eclosion peak of all populations. Day 12 represents the fecundity peak of the populations (unpublished data from the lab). Day 22 was chosen as the mid-point between early and late life.

2.4.2. To test the contribution of high early-life reproduction on decrease in mid-life reproductive output in populations that consumed high protein adult diet

Based on the result that in case of varying developmental diet and high protein adult diet, there exists a tradeoff in reproductive output between early-life and mid-life (Figure 1A; Table 1), we hypothesized that high early-life reproduction causes the decrease in mid-life reproductive output in populations that were reared in HH dietary regime as opposed to LH dietary regime. To test this hypothesis, we reared the flies from the high protein adult diets (HH and LH) as virgins for 20 days to remove the effect of early-life reproduction, and then allowed them to mate and reproduce in mid-life. We then count the number of viable progeny produced in mid-life when the effect of early-life reproduction has been minimized and thus equalized.

If mid-life reproductive output does trade-off with early-life reproductive output, then the difference between HH and LH in reproductive output in mid-life should no longer exist when investment into reproductive output in early-life has been minimized.

2.4.3. Understanding the physiological basis of the differential impact of development and adult diets on age-dependent reproductive output

To understand how developmental and adult nutrition interact across age to influence reproductive ability, we measured the size of ovaries, counted the number of ovarioles, and quantified the efficiency of maturation of egg chambers in flies reared in HH, HL, LH and LL diets in early, mid and late stages of adult life i.e. on days 10, 12, 22 and 32 from the day of egg collection. In total, for these experiments, we dissected 1,280 females for the three assays – 30 females x 4 dietary regimes x 4 age-points for measuring ovary size, 30 females x 4 dietary regimes x 4 age-points for quantifying the

number of ovarioles, and 20 females x 4 dietary regimes x 4 age-points for quantifying egg chamber stages.

2.4.4. To test the contribution of development time on the effect of developmental diet on reproductive output in early-life

Flies reared in low protein diet experience a development delay of approximately 24 hours as compared to the flies reared in high protein diet (Mudunuri *et al.*, 2024). A delay in development implies potential difference in reproductive maturity of flies, especially very early in adult life (Markow, 1996). Thus, we suspected that the effect of developmental diet on reproductive output in early-life seen in previous experiments is driven by differences in reproductive maturity due to differences in developmental time. To test this hypothesis, we conducted a pilot experiment in which we counted the number of viable progeny produced by flies reared in HH, HL, LH and LL diets post matching adult age so as to minimize the differences in reproductive maturity caused by developmental delay. The assay was set up approximately a day post-eclosion for all the treatments.

2.5. Statistical analysis

2.5.1. The effect of developmental and adult diets on age-dependent reproductive output

To understand how developmental and adult nutrition interact across age to influence reproductive output, we counted the number of viable progeny produced by flies reared in HH, HL, LH and LL diets on days 10, 12, 22 and 32 from the day of egg collection. We used generalised linear models (GLMs) with negative binomial error structure and log link function to investigate the relationship between the three fixed factors (with respective levels) – developmental diet (H and L), adult diet (H and L), age (10, 12, 22, 32), and the response variable – viable progeny count. When the interaction effect was significant, post-hoc was performed using Tukey's HSD test.

To understand how the different combinations of developmental and adult diets compare with one another, we calculated effect size using Cohen's d (Cohen, 1988) for all treatment comparisons within each age point.

2.5.2. The contribution of high early-life reproduction on decrease in mid-life reproductive output in populations that consumed high protein adult diet

To test whether decrease in mid-life reproductive output is due to greater early-life reproduction in populations that consumed a high protein adult diet, we counted the number of viable progeny in flies reared as virgins in early-life in HH and LH dietary regimes, and allowed them to mate and reproduce only in mid-life. We used generalised linear models (GLMs) with negative binomial error structure and log link function to investigate the relationship between the fixed factor (with respective levels) – treatments (HH and LH), and the response variable – viable progeny count.

2.5.3. Physiological basis of differential reproductive output

2.5.3.1. Ovary size

To understand how developmental and adult nutrition interact across age to affect ovary size, we measured the size of ovaries in flies reared in HH, HL, LH and LL diets on days 10, 12, 22 and 32 from the day of egg collection. We used generalised linear models (GLMs) with Gamma error structure and identity link function to investigate the relationship between the three fixed factors (with respective levels) – developmental diet (H and L), adult diet (H and L), age (10, 12, 22, 32), and the response variable – ovary size. When the interaction effect was significant, post-hoc was performed using Tukey's HSD test.

2.5.3.2. Number of ovarioles

To understand how developmental and adult nutrition interact across age to affect the number of ovarioles, we counted the number of ovarioles in flies reared in HH, HL, LH and LL diets on days 10, 12, 22 and 32 from the day of egg collection. We used generalised linear models (GLMs) with negative binomial error structure and log link function to investigate the relationship between the three fixed factors (with respective levels) – developmental diet (H and L), adult diet (H and L), age (10, 12, 22, 32), and the response variable – number of ovarioles.

2.5.3.3. Maturation of egg chambers

To understand how developmental and adult nutrition interact across age to affect the number of egg chambers, we counted the number of egg chambers in flies reared in HH, HL, LH and LL diets on days 10, 12, 22 and 32 from the day of egg collection. We used generalised linear models (GLMs) with negative binomial error structure and log link function to investigate the relationship between the three fixed factors (with respective levels) – developmental diet (H and L), adult diet (H and L), age (10, 12, 22, 32), and the response variable – number of egg chambers. Post-hoc was performed using Tukey's HSD test.

To understand how developmental and adult nutrition interact across age to affect maturation of egg chambers, we calculated the proportion of egg chambers that undergo vitellogenesis (i.e. number of egg chambers post vitellogenesis/ total number of egg chambers) in flies reared in HH, HL, LH and LL diets on days 10, 12, 22 and 32 from the day of egg collection. We used generalised linear models (GLMs) with gamma error structure and log link function to investigate the relationship between the three fixed factors (with respective levels) – developmental diet (H and L), adult diet (H and L), age (10, 12, 22, 32), and the response variable – proportion of egg chambers that undergo vitellogenesis. When the interaction effect was significant, post-hoc was performed using Tukey's HSD test.

2.5.4. The contribution of development time on the effect of developmental diet on reproductive output in early-life

To test whether the effect of developmental diet on reproductive output in early-life was driven by differences in development delay and the subsequent differences in reproductive maturity in early life, we counted the number of viable progeny produced by adult age matched flies reared in HH, HL, LH and LL dietary regimes, approximately a day post eclosion. We used generalised linear models (GLMs) with negative binomial error structure and log link function to investigate the relationship between the fixed factor (with respective levels) – treatments (HH, HL, LH and LL), and the response variable – viable progeny count.

All data was analyzed using R 4.3.2; and all plots were created in Python 3.11 using Spyder Version 5.

Chapter 3 Results

3.1. The effect of developmental and adult diets on viable progeny produced is dependent on age

Effect of developmental diet on the number of viable progeny produced varies with age ($p < 0.01$; Table S1). In early-life, populations reared in a high protein developmental diet produce more progeny as compared to populations reared in low protein developmental diet ($p < 0.01$; Figure 1A; Table S2). However, in mid-life, populations reared in a low protein developmental diet produce more progeny as compared to populations reared in a high protein developmental diet ($p = 0.01$; Figure 1A; Table S2). In late-life, developmental diet has no effect on the number of progeny produced ($p = 0.64$; Figure 1A; Table S2).

There appears to exist a tradeoff between early-life and mid-life reproductive output. Populations that are reared in high protein developmental diet have higher reproductive output in early-life and have lower reproductive output in mid-life in comparison to populations reared in low protein developmental diet (Figure 1A; Table S2). Populations that are reared in low protein developmental diet have lower reproductive output in early-life and have higher reproductive output in mid-life in comparison to populations reared in high protein developmental diet (Figure 1A; Table S2).

Effect of adult diet on the number of viable progeny produced varies with age ($p < 0.01$; Table S1). Populations reared in high protein adult diet produce more progeny than populations reared in low protein adult diet at all measured age points, except in very early life i.e. 10th day from egg collection (Figure 1B; Table S3).

3.2. The differential effect of developmental diet across age on viable progeny produced is greater when adult diet is high in protein

By looking at the effect size of treatment comparisons within each age point, we see that the tradeoff between early-life and mid-life reproductive output when developmental diet is changed is driven by populations reared in high protein adult diet rather than by populations reared in low protein adult diet (i.e. although in early-life HH > LH and HL > LL with large effect size, in mid-life LH > HH with medium effect size and LL > HL with small effect size; Table 1).

3.3. High early-life reproduction is not the cause for decrease in mid-life reproductive output in populations that consume high protein adult diet

To test whether the above mentioned decrease in mid-life reproductive output is due to a tradeoff with investment into early-life reproductive output in HH versus LH i.e. varying developmental diet but high protein adult diet (Table 1; Figure 1A), we minimized the investment into reproduction in these populations in early-life by rearing them as virgins throughout early-life, and allowed them to mate and reproduce only in mid-life. The viable progeny produced in mid-life in these experimental populations were counted to check whether or not difference in mid-life reproductive output between HH and LH exists since their investment into early-life reproduction has been minimized. From Figure 1C, we see that the difference between HH and LH still exists in mid-life despite being reared as virgins in early-life ($p < 0.01$).

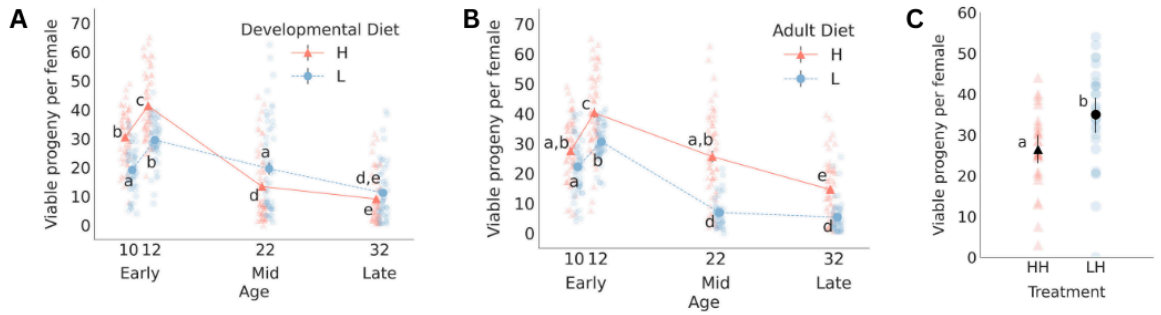

Figure 1. (A) Representation of the effect of developmental diet across age on the number of viable progeny produced. (B) Representation of the effect of adult diet across age on the number of viable progeny produced. (C) Representation of the number of viable progeny produced by populations reared as virgins on HH and LH dietary regimes in early-life and allowed to mate and reproduce in mid-life. The red triangle marker with solid line represents high protein diet H; the blue circle marker with dotted line represents low protein diet L. The black solid vertical lines indicate the standard error for each treatment. Some error bars are not visible because of their small size. Different lower-case letters denote statistically significant differences ($p < 0.05$).

Table 1. Effect sizes of the number of viable progeny produced compared between the four dietary treatments (HH, HL, LH and LL) at each measured age (10, 12, 22 and 32)

Age	Groups	Mean difference	Effect size (Cohen's d)	Interpretation
10	HH-LH	11.5	1.340800264	Large
10	HH-HL	5.483333333	0.643431035	Medium
10	HH-LL	16.75057471	2.019201605	Large
10	LH-HL	-6.016666667	0.776498273	Medium
10	LH-LL	5.250574713	0.701570741	Medium
10	HL-LL	5.250574713	1.518519955	Large
12	HH-LH	19.15	2.159882463	Large
12	HH-HL	17.03333333	2.250573132	Large
12	HH-LL	21.5	2.896495465	Large
12	LH-HL	-2.116666667	0.289920494	Small
12	LH-LL	2.35	0.328684077	Small
12	HL-LL	4.466666667	0.818368135	Large
22	HH-LH	-9.85	0.726714491	Medium
22	HH-HL	14.66666667	1.593205791	Large
22	HH-LL	12.84285714	1.364598283	Large
22	LH-HL	24.51666667	2.261217869	Large
22	LH-LL	22.69285714	2.049853936	Large
22	HL-LL	-1.823809524	0.407290118	Small
32	HH-LH	-3.556896552	0.371849854	Small
32	HH-HL	7.737547893	1.029738235	Large
32	HH-LL	7.11453202	0.955326278	Large
32	LH-HL	11.29444444	1.38896081	Large
32	LH-LL	10.67142857	1.323958452	Large
32	HL-LL	-0.623015873	0.118226957	non-significant

3.4. The effect of developmental and adult diets on ovary size is dependent on age

Effect of developmental diet on ovary size varies with age ($p < 0.01$; Table S4). In very early life, populations reared in a high protein developmental diet have larger ovaries as compared to populations reared in low protein developmental diet ($p < 0.01$; Figure 2A; Table S5). However, in mid-life, populations reared in a low protein developmental diet have larger ovaries as compared to populations reared in a high protein developmental diet ($p = 0.04$; Figure 2A; Table S5). In late-life, developmental diet has no effect on ovary size ($p = 0.89$; Figure 2A; Table S5).

Effect of adult diet on ovary size varies with age ($p < 0.01$; Table S4). Populations reared in high protein adult diet have larger ovaries than populations reared in low protein adult diet at all measured age points, except in early life (Figure 2B; Table S6).

3.5. High protein developmental diet increases the number of ovarioles

Irrespective of adult diet and age, populations reared in high protein developmental diet have a greater number of ovarioles ($p = 0.02$; Figure 2C; Table S7). The number of ovarioles do not vary due to adult diet ($p = 0.06$; Figure 2D; Table S7). There is also no change in the number of ovarioles with age in our measured age-points ($p = 0.16$; Table S7).

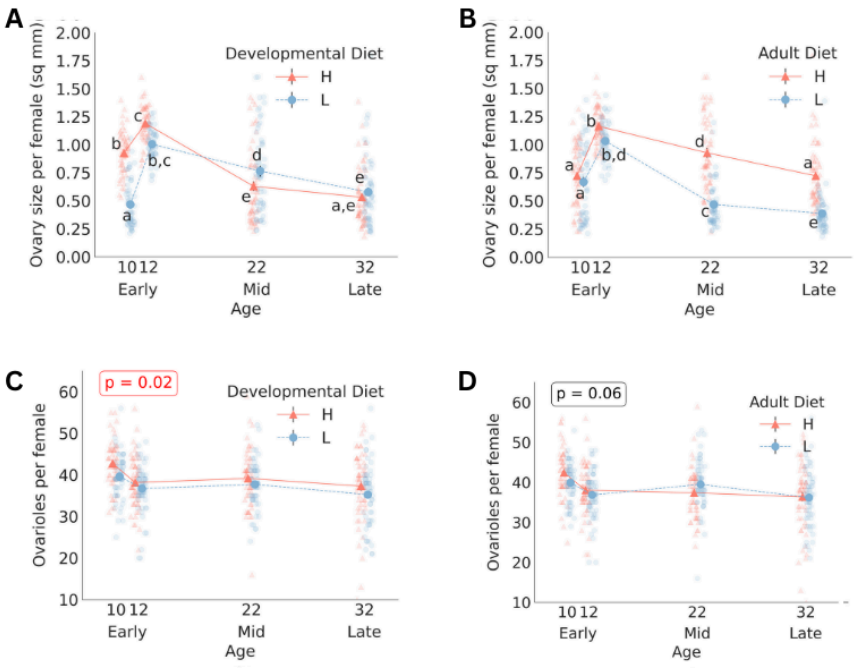

Figure 2. (A) Representation of the effect of developmental diet across age on ovary size. (B) Representation of the effect of adult diet across age on ovary size. (C) Representation of the effect of developmental diet across age on the number of ovarioles. (B) Representation of the effect of adult diet across age on the number of ovarioles. The red triangle marker with solid line represents high protein diet H; the blue circle marker with dotted line represents low protein diet L. The black solid vertical lines indicate the standard error for each treatment. Some error bars are not visible because of their small size. Different lower-case letters in (A) and (B) denote statistically significant differences ($p < 0.05$). In (C) and (D), interaction effect is not significant. Therefore, p-value of the main effect of developmental and adult diets have been presented directly in the respective graphs.

3.6. Total number of egg chambers in ovaries varies with age, but is independent of diet

The number of egg chambers vary with age ($p < 0.01$; Figure 3C; Table S8); after the initial increase in number in early-life, the number of egg chambers decreases with age (Figure 3C; Table S9). However, the number of egg chambers do not vary in response to either developmental diet ($p = 0.07$; Table S8) or adult diet ($p = 0.25$; Table S8).

3.7. The effect of developmental and adult diets on the proportion of egg chambers that undergo vitellogenesis is dependent on age

Effect of developmental diet on proportion of egg chambers that undergo vitellogenesis varies with age ($p < 0.01$; Table S10). In very early life, populations reared in a high protein developmental diet have a greater proportion of egg chambers that undergo vitellogenesis as compared to populations reared in a low protein developmental diet ($p < 0.01$; Figure 3D; Table S11). However, we find that developmental diet has no effect on the proportion of egg chambers that undergo vitellogenesis at any other age-points (Figure 3D; Table S11).

Effect of adult diet on proportion of egg chambers that undergo vitellogenesis varies with age ($p < 0.01$; Table S10). Populations reared in high protein adult diet have greater proportion of egg chambers that undergo vitellogenesis than populations reared in low protein adult diet at all measured age points, except in early life (Figure 3E; Table S12).

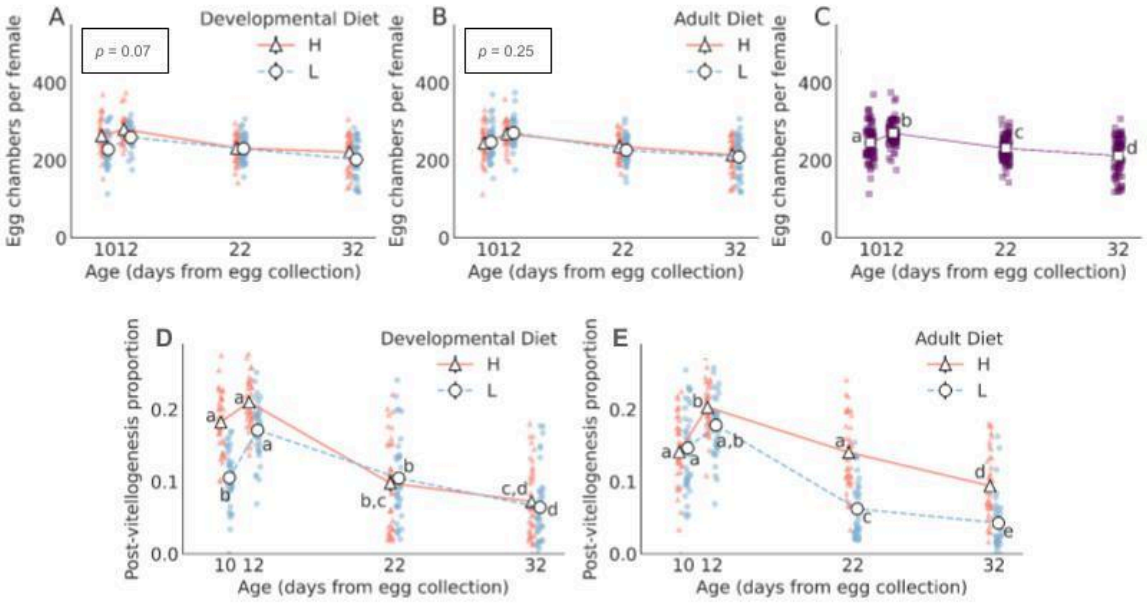

Figure 3. (A) Representation of the effect of developmental diet across age on the total number of egg chambers. (B) Representation of the effect of adult diet across age on the total number of egg chambers. (C) Representation of the change in the total number of egg chambers with age, irrespective of differences in developmental and adult diets. (D) Representation of the effect of developmental diet across age on the proportion of egg chambers that are in stages >= 8 i.e. egg chambers that undergo vitellogenesis. (E) Representation of the effect of adult diet across age on the proportion of egg chambers that are in stages >= 8 i.e. egg chambers that undergo vitellogenesis. The red triangle marker with solid line represents high protein diet H; the blue circle marker with dotted line represents low protein diet L. The black solid vertical lines indicate the standard error for each treatment. Some error bars are not visible because of their small size. Different lower-case letters in (C), (D) and (E) denote statistically significant differences ($p < 0.05$). In (A) and (B), the interaction effect is not significant. Therefore, p-value of the main effect of developmental and adult diets have been presented directly in the respective graphs.

3.8. The effect of developmental diet on reproductive output in early-life is potentially contributed by difference in reproductive maturity due to varying developmental time

When the number of viable progeny produced by adult age-matched populations are counted to minimize the differences in reproductive maturity due to changes in developmental time, we see that the effect of developmental diet on reproductive out varies based on adult diet in early life ($p < 0.01$; Figure 4; Table S13). When adult diet is high in protein, populations reared in low protein developmental diet have more number of viable offsprings as compared to populations reared in high

protein developmental diet ($p = 0.03$; Figure 4; Table S13). When adult diet is low in protein, populations reared in high protein developmental diet have more number of viable offsprings as compared to populations reared in low protein developmental diet ($p < 0.01$; Figure 4; Table S13).

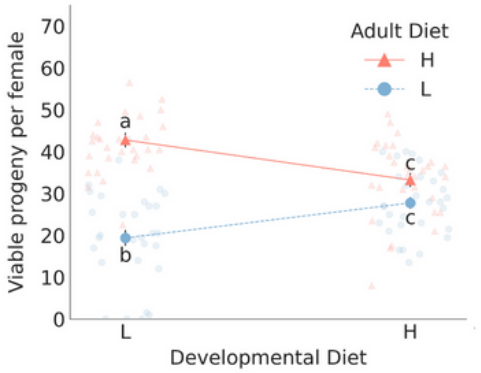

Figure 4. Representation of the effect of developmental and adult diets on the number of viable progeny produced approximately one-day post-eclosion. Developmental diets are represented on the x-axis and the adult diets are represented as lines. The red solid line with triangle marker represents the high protein adult diet, and the blue dotted line with circle marker represents the low protein adult diet. The black solid vertical lines indicate the standard error for each treatment. Some error bars are not visible because of their small size. Different lower-case letters denote statistically significant differences ($p < 0.05$).

Chapter 4 Discussion

In this study, we see that the effect of developmental and adult diets on reproductive output of populations vary with age (Figure 1A; Figure 1B). Overall, there is a gradual shift in reproductive output being affected only by developmental diet in very-early-life to reproductive output being affected by both development and adult diets in early-life and mid-life to reproductive output being affected only by adult diet in late-life (Figure 1A; Figure 1B). This observation is also in-line with results from studies that have looked at the effect of developmental diet across life, which show that the impact of development diet on reproductive output and gene expression is large in early-life, but minimal in late-life (May *et al.*, 2015, 2021).

In very-early-life, reproductive output is dictated only by developmental diet, where we see that populations reared on high protein developmental diet produce more viable progeny than populations reared on low protein developmental diet (Figure 1A). In early-life, reproductive output is dictated by both developmental and adult diets, where we see that populations reared in high protein developmental diet and high protein adult diet produce more viable progeny than populations reared in low protein developmental diet and low protein adult diet, respectively (Figure 1A; Figure 1B). Thus, in early-life, the effect of developmental diet on reproductive output is in-line with the silver spoon hypothesis. In mid-life, however, although reproductive output is dictated by both developmental and adult diets, we see that populations reared in low protein developmental diet and high protein adult diet produce more viable progeny than populations reared in high protein developmental diet and low protein adult diet, respectively (Figure 1A; Figure 1B). Thus, in mid-life, the effect of developmental diet on reproductive output does not agree with the silver spoon, the thrifty phenotype or the PAR hypotheses. In late-life, we see that reproductive output is dictated only by adult diet, where populations reared in high protein adult diet produce more viable progeny than populations reared in low protein adult diet (Figure 1B). Thus, we see a clear age-specific pattern in how different theories that attempt to explain the effect of developmental diet on reproductive output are true (or false) at different ages. A recent study that examined the age-specific reproductive success in a natural population of collared flycatcher females too found similar results (Spagopoulou *et al.*, 2020).

One of the major components of the above results reveal that in early-life, populations that consume high protein diet during developmental stage have *higher* reproductive output as opposed to those that consumed low protein diet during their developmental stage; however, in mid-life, populations that consume high protein diet during developmental stage have *lower* reproductive output as opposed to those that consumed low protein diet during their development stage (Figure 1A). This kind of early-late life trade-off in reproductive ability is central to life-history theories of aging like William's antagonistic pleiotropy that explain the evolution of aging (Williams, 1957). Even though the correlation between early and late life reproductive trade-off has been seen several times, we do not understand the mechanistic basis of this trade-off. A few studies have suggested that increased early-life reproduction itself is the cause of the decreased reproductive output in late-life (Nussey *et al.*, 2006; McKenna-Ell *et al.*, 2023). To test this hypothesis in my experimental framework, I maintained the individuals from the populations reared in HH and LH dietary regimes as virgins in early-life so as to minimize and thereby equalize investment into reproduction in early-life between the two groups. When allowed to mate and produce progeny in mid-life, if both populations have an equal number of progeny, it would imply that the decrease in reproductive output in mid-life in populations reared in HH dietary regime is indeed due to investment into reproduction in early-life; however, if the difference in reproductive output between the populations reared in HH and LH dietary regimes persisted, then it would imply that investment into early reproduction had little or no role in decreased reproductive output in mid-life. From Figure 1C, we see that the difference in reproductive output between HH and LH persists! Therefore, increased early-life reproductive output is not the cause for decreased reproductive output in mid-life for populations reared in HH versus LH

dietary regimes. Hence, developmental diet's effect on mid-life reproductive output is not via early-life reproductive investment.

An alternative possible explanation for how developmental diets can influence reproductive output in an age-dependent manner in adult life is via structures that do not undergo histolysis during pupation and are hence carried over from larva to adults. For example, in case of fruit flies, larval fat body, malpighian tubules, some gut tissues and a few neurons are not histolysed during pupation (Riddiford, 1993; Lee *et al.*, 1999; Klapper, 2000; Nelliot *et al.*, 2006; Aguila *et al.*, 2007, 2013). It is possible that developmental diets regulate age-dependent reproductive output via these structures, particularly the larval fat body. Fat bodies are known to affect oogenesis via regulation of fat metabolism (Sieber and Spradling, 2015) and synthesis of yolk protein 3, which is taken up by oocytes during vitellogenesis (Bownes and Reid, 1990). The time-dependent presence of the larval fat body in the adult also makes it a strong candidate via which developmental diet regulates adult traits in an age-dependent manner. The larval fat body is present for only about a week in early-life of the adult (Aguila *et al.*, 2007, 2013). This exhaustion of the larval fat body coincides with the mid-life decrease in the number of progeny produced by populations reared in high protein developmental diet (Figure 1A). Therefore, considering the role of fat body in regulating oogenesis as well as the timing of disappearance of the larval fat body in the adult, it is likely that developmental diet regulates oogenesis via the fat body. Further studies are required to test this and to identify the exact mechanism of the age-dependent effect of developmental diet on adult reproductive output.

From the results, it is also important to note that although the number of viable progeny produced by the populations are dependent on both the developmental and adult diets, the various physiological processes involved in egg production are not necessarily dependent on both the diets. We see that the number of ovarioles present in the ovaries is only dependent on developmental diet (Figure 2C; Figure 3A); while the ovary size and the proportion of egg chambers that undergo vitellogenesis are dependent on both developmental and adult diets (Figure 2A; Figure 2B; Figure 3D; Figure 3E). This implies that developmental diet affects production of ovarioles; whereas, both developmental and adult diets regulate the process of vitellogenesis.

In *D. melanogaster*, ovaries are formed in the late larval and early pupal stages (Kerkis, 1931; King, 1970). Each ovary contains about 18 egg-chamber-producing tubules called ovarioles. The number of ovarioles formed during development are dependent on the number of terminal filament (TF) cells present in the larva (Green and Extavour, 2014). The number of TF is dependent on the number of somatic gonad precursors (SGP cells) whose numbers are regulated by the expression of Insulin Receptor (InR), which is an integral part of the larger nutrient-sensing pathways (Green and Extavour, 2014). Therefore, to understand the effect of developmental and adult diets on ovariole formation, I quantified the number of ovarioles present in the ovaries of adult females reared in different dietary regimes. The results show that more ovarioles are formed in females reared in high protein developmental diet, irrespective of adult diet. This could imply that a high protein developmental diet increases the number of SGP cells and TF cells in the larva resulting in an increase in the number of ovarioles formed.

The anterior tip of the ovariole contains the germanium in which the germ stem cells (GSCs) are present. The GSCs divide asymmetrically to give rise to egg chambers. As the egg chambers leave the germarium and continue to move within the ovariole from the anterior tip to the posterior end, they grow in size, mature and become competent for fertilization. Based on morphology, these egg chambers are classified into 14 stages. The shift from stage 7 to stage 8 is marked by vitellogenesis – the initiation of increased accumulation of yolk by the oocyte (Cummings and King, 1969; King, 1970). Yolk proteins synthesized in the fat body are secreted into the hemolymph and taken up by the oocyte during vitellogenesis (Bownes *et al.*, 1993). Malnourishment is known to reduce transcription of yolk protein in the fat body, decreasing the number of egg chambers that can undergo vitellogenesis (Bownes, 1989). Failure to undergo vitellogenesis results in the termination of the egg chambers (Terashima and Bownes, 2006), which ultimately reduces the reproductive output of the individual. Thus vitellogenesis acts as one of the major checkpoints in the process of oogenesis that is regulated

by nutrient availability. From my results, it is evident that the process of vitellogenesis is affected by both developmental and adult diets (Figures 3D and 3E). Populations reared in high protein developmental diet have a greater proportion of egg chambers that undergo vitellogenesis only in very-early-life; developmental diet does not significantly affect vitellogenesis post that (Figure 3D). Populations reared in high protein adult diet have greater proportion of egg chambers that undergo vitellogenesis in mid-life and late-life (Figure 3E). Overall, there is a gradual shift from developmental diet affecting vitellogenesis in very-early-life to adult diet affecting vitellogenesis in mid-life and late-life. This shift could be regulated via the fat body since the adult flies eclose with the larval fat body which gets completely replaced by the adult fat body within a week (Aguila *et al.*, 2007, 2013).

Additionally, the effect of developmental diet on reproductive output in very-early-life could also be because of differences in reproductive maturity due to varying developmental time. A delay in development implies potential difference in reproductive maturity of flies, especially very early in adult life (Markow, 1996). Since populations reared in low protein developmental diet take longer to eclose (Mudunuri *et al.*, 2024), it is possible that in the previous experiments, the effects seen in very-early-life are influenced by this developmental time difference. From Figure 4, it is evident that when the effect of developmental delay is minimized, the effect of developmental diet on reproductive output in early-life is dependent on the adult diet. When adult diet is high in protein, populations reared in low protein developmental diet produce more number of progeny than populations reared in high protein developmental diet; while, when adult diet is low in protein, populations reared in high protein developmental diet produce more number of progeny than populations reared in low protein developmental diet. However, this result is from a preliminary pilot study of age-matched individuals and should therefore be taken with a pinch of salt. Further experiments are needed to understand this better.

Overall, the results from this thesis show that the effects of developmental and adult diets on reproductive output vary with age (Figure 1A and 1B). It also shows that mid-life decrease in reproductive output is not caused by high reproductive output in early-life (Figure 1C). This age-dependent effect of developmental diet of adult reproductive output could be via the larval fat body. Additionally, the results highlight that although the number of progeny produced is dependent on the effect of both developmental and adult diets across age, the various physiological processes involved in egg production are not necessarily dependent on both the diets (Figure 2; Figure 3). These results highlight the importance of studying adult traits in an age-specific manner.

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Appendix

NOTE: Red highlight indicates statistical significance i.e p < 0.05

Table S1. Statistics of viable progeny count analysis

	Sum Sq	Df	F values	Pr(>F)
Age	184.1222681	3	55.66035221	0
Larva_diet	18.66780657	1	16.92989174	4.60459E-05
Adult_diet	5.159319551	1	4.679002921	0.031053548
Age:Larva_diet	20.18624794	3	6.102323646	0.000446604
Age:Adult_diet	71.75352864	3	21.69116597	0
Larva_diet:Adult_diet	0.330722305	1	0.299933085	0.584192919
Age:Larva_diet:Adult_diet	5.526270316	3	1.670597236	0.172541313
Residuals	501.7074013	455		

Table S2. Statistics of viable progeny count Tukey analysis for developmental diet x age

	contrast	estimate	standard error	z.ratio	p.value
1	Age10 C - Age12 C	-0.443863801	0.083735367	-5.30079242	3.19802E-06
2	Age10 C - Age22 C	0.200187181	0.089182075	2.244701984	0.324882155
3	Age10 C - Age32 C	0.675117247	0.092877947	7.268864884	0
4	Age10 C - Age10 H	-0.472813573	0.083649719	-5.652303179	4.41165E-07
5	Age10 C - Age12 H	-0.759396465	0.082869338	-9.163781042	0
6	Age10 C - Age22 H	0.526864366	0.09071943	5.807624332	1.76808E-07
7	Age10 C - Age32 H	0.855884051	0.095663447	8.946824272	0
8	Age12 C - Age22 C	0.644050982	0.086977208	7.40482476	0
9	Age12 C - Age32 C	1.118981048	0.090762907	12.32861629	0
10	Age12 C - Age10 H	-0.028949772	0.081294943	-0.356107907	0.999965912
11	Age12 C - Age12 H	-0.315532664	0.080491734	-3.92006293	0.002254234
12	Age12 C - Age22 H	0.970728167	0.088552849	10.96213366	0
13	Age12 C - Age32 H	1.299747852	0.093611363	13.88450941	0
14	Age22 C - Age32 C	0.474930066	0.095810938	4.956950364	1.96968E-05
15	Age22 C - Age10 H	-0.673000753	0.086894755	-7.745010102	0
16	Age22 C - Age12 H	-0.959583646	0.086143775	-11.13932657	0
17	Age22 C - Age22 H	0.326677186	0.093719997	3.485672183	0.011548899
18	Age22 C - Age32 H	0.65569687	0.098513542	6.655905926	7.87992E-10
19	Age32 C - Age10 H	-1.14793082	0.090683896	-12.65859622	0
20	Age32 C - Age12 H	-1.434513712	0.089964552	-15.94532163	0
21	Age32 C - Age22 H	-0.14825288	0.097243552	-1.524552299	0.794386886
22	Age32 C - Age32 H	0.180766804	0.101871432	1.774460233	0.637547716
23	Age10 H - Age12 H	-0.286582892	0.080402631	-3.564347203	0.008732977
24	Age10 H - Age22 H	0.999677939	0.088471865	11.29938817	0
25	Age10 H - Age32 H	1.328697624	0.093534758	14.20538895	0
26	Age12 H - Age22 H	1.286260831	0.087734386	14.66085185	0
27	Age12 H - Age32 H	1.615280516	0.092837507	17.39900793	0
28	Age22 H - Age32 H	0.329019684	0.099907409	3.293246082	0.022162905

Table S3. Statistics of viable progeny count Tukey analysis for adult diet x age

	contrast	estimate	standard error	z.ratio	p.value
1	Age10 L - Age12 L	-0.355006402	0.083132234	-4.270382053	0.00051665
2	Age10 L - Age22 L	1.133414028	0.094756244	11.96136507	0
3	Age10 L - Age32 L	1.384181651	0.100087776	13.82967733	0
4	Age10 L - Age10 H	-0.228424913	0.083649719	-2.730731383	0.113381314
5	Age10 L - Age12 H	-0.603865204	0.082472419	-7.322026089	0
6	Age10 L - Age22 H	-0.161973822	0.083895417	-1.930663523	0.529561226
7	Age10 L - Age32 H	0.391208306	0.087143414	4.48924696	0.000192386
8	Age12 L - Age22 L	1.48842043	0.09303744	15.99808028	0
9	Age12 L - Age32 L	1.739188053	0.098462086	17.66353045	0
10	Age12 L - Age10 H	0.126581489	0.081697582	1.549390889	0.780374472
11	Age12 L - Age12 H	-0.248858802	0.080491734	-3.091731147	0.041731159
12	Age12 L - Age22 H	0.19303258	0.081949133	2.355517053	0.263620844
13	Age12 L - Age32 H	0.746214708	0.085271296	8.751065595	0
14	Age22 L - Age32 L	0.250767623	0.108455335	2.312174148	0.286711499
15	Age22 L - Age10 H	-1.361838941	0.093500119	-14.5651038	0
16	Age22 L - Age12 H	-1.737279233	0.092448346	-18.79189088	0
17	Age22 L - Age22 H	-1.29538785	0.093719997	-13.82189387	0
18	Age22 L - Age32 H	-0.742205722	0.096638354	-7.68023972	0
19	Age32 L - Age10 H	-1.612606564	0.098899391	-16.30552579	0
20	Age32 L - Age12 H	-1.988046855	0.097905638	-20.30574443	0
21	Age32 L - Age22 H	-1.546155473	0.09910729	-15.60082482	0
22	Age32 L - Age32 H	-0.992973345	0.101871432	-9.747319045	0
23	Age10 H - Age12 H	-0.375440291	0.081026085	-4.633573161	9.75528E-05
24	Age10 H - Age22 H	0.066451091	0.082474041	0.805721294	0.992861404
25	Age10 H - Age32 H	0.619633219	0.085775877	7.223863404	0
26	Age12 H - Age22 H	0.441891383	0.081279714	5.436674918	1.5089E-06
27	Age12 H - Age32 H	0.995073511	0.08462816	11.75818437	0
28	Age22 H - Age32 H	0.553182128	0.086015502	6.431191057	3.54186E-09

Table S4. Statistics of ovary size analysis

	Sum Sq	Df	F values	Pr(>F)
Age	15.81344618	3	49.24674166	0
Larva_diet	10.80655468	1	100.9623583	0
Adult_diet	1.387885795	1	12.96659547	0.000351827
Age:Larva_diet	11.0713314	3	34.47869559	0
Age:Adult_diet	3.08851079	3	9.618339433	3.61259E-06
Larva_diet:Adult_diet	0.393838012	1	3.679508932	0.055707863
Age:Larva_diet:Adult_diet	0.206260416	3	0.642342807	0.588101666
Residuals	48.91521525	457		

Table S5. Statistics of ovary size Tukey analysis for developmental diet x age

	contrast	estimate	standard error	t.ratio	p.value
1	Age10 L - Age12 L	-0.535901355	0.048194261	-11.11960925	0
2	Age10 L - Age22 L	-0.298442593	0.039373238	-7.579833539	0
3	Age10 L - Age32 L	-0.111477778	0.032562916	-3.423458031	0.015406364
4	Age10 L - Age10 H	-0.457814815	0.043879012	-10.43357171	0
5	Age10 L - Age12 H	-0.724552235	0.055537533	-13.0461725	0
6	Age10 L - Age22 H	-0.162144445	0.034462116	-4.705005527	9.11329E-05
7	Age10 L - Age32 H	-0.065131482	0.030685204	-2.122569618	0.401885001
8	Age12 L - Age22 L	0.237458763	0.055462915	4.281397087	0.000594551
9	Age12 L - Age32 L	0.424423578	0.050854956	8.345766264	0
10	Age12 L - Age10 H	0.07808654	0.058747346	1.32919264	0.887420828
11	Age12 L - Age12 H	-0.18865088	0.067899194	-2.778396435	0.103019142
12	Age12 L - Age22 H	0.373756911	0.052091462	7.175012828	0
13	Age12 L - Age32 H	0.470769874	0.049673582	9.477268441	0
14	Age22 L - Age32 L	0.186964815	0.042588633	4.390016851	0.000373485
15	Age22 L - Age10 H	-0.159372222	0.051757277	-3.079223448	0.045275139
16	Age22 L - Age12 H	-0.426109642	0.06195051	-6.878226565	5.55871E-10
17	Age22 L - Age22 H	0.136298148	0.044057752	3.09362466	0.043402572
18	Age22 L - Age32 H	0.233311111	0.04117074	5.666915646	7.16498E-07
19	Age32 L - Age10 H	-0.346337037	0.046785761	-7.402616367	0
20	Age32 L - Age12 H	-0.613074457	0.057861536	-10.5955442	0
21	Age32 L - Age22 H	-0.050666667	0.038094319	-1.330032088	0.887086402
22	Age32 L - Age32 H	0.046346296	0.034714859	1.335056428	0.885072199
23	Age10 H - Age12 H	-0.26673742	0.064907484	-4.109501789	0.001212357
24	Age10 H - Age22 H	0.29567037	0.048126929	6.143553671	4.90389E-08
25	Age10 H - Age32 H	0.392683333	0.045498853	8.630620538	0
26	Age12 H - Age22 H	0.562407791	0.058951261	9.540216449	0
27	Age12 H - Age32 H	0.659420754	0.056826011	11.60420631	0
28	Age22 H - Age32 H	0.097012963	0.036502265	2.657724468	0.138549809

Table S6. Statistics of ovary size Tukey analysis for adult diet x age

	contrast	estimate	standard error	t.ratio	p.value
1	Age10 L - Age12 L	-0.36214133	0.054382162	-6.659193359	2.21108E-09
2	Age10 L - Age22 L	0.199485185	0.036366396	5.485426273	1.89686E-06
3	Age10 L - Age32 L	0.279646296	0.034567372	8.08989162	0
4	Age10 L - Age10 H	-0.057007407	0.043879012	-1.299195339	0.898976183
5	Age10 L - Age12 H	-0.497504853	0.059207314	-8.402759996	0
6	Age10 L - Age22 H	-0.259264815	0.049711123	-5.215428672	7.67917E-06
7	Age10 L - Age32 H	-0.055448148	0.043160667	-1.284691629	0.904287348
8	Age12 L - Age22 L	0.561626515	0.049267731	11.39948007	0
9	Age12 L - Age32 L	0.641787626	0.047955164	13.38307644	0
10	Age12 L - Age10 H	0.305133922	0.055046909	5.543161786	1.39568E-06
11	Age12 L - Age12 H	-0.135363524	0.067899194	-1.993595427	0.487323684
12	Age12 L - Age22 H	0.102876515	0.059799584	1.720355025	0.673963429
13	Age12 L - Age32 H	0.306693182	0.054476029	5.629874038	8.75896E-07
14	Age22 L - Age32 L	0.080161111	0.025783581	3.108998421	0.041477215
15	Age22 L - Age10 H	-0.256492593	0.037353145	-6.866693325	5.98445E-10
16	Age22 L - Age12 H	-0.696990038	0.054547189	-12.7777443	0
17	Age22 L - Age22 H	-0.45875	0.044057752	-10.41246952	0
18	Age22 L - Age32 H	-0.254933333	0.036506616	-6.983209125	2.82037E-10
19	Age32 L - Age10 H	-0.336653704	0.035604015	-9.455498193	0
20	Age32 L - Age12 H	-0.77715115	0.053364636	-14.56303675	0
21	Age32 L - Age22 H	-0.538911111	0.042584903	-12.65498032	0
22	Age32 L - Age32 H	-0.335094445	0.034714859	-9.652766847	0
23	Age10 H - Age12 H	-0.440497446	0.059818465	-7.363904192	0
24	Age10 H - Age22 H	-0.202257407	0.05043747	-4.010062487	0.001806598
25	Age10 H - Age32 H	0.001559259	0.043995294	0.035441501	1
26	Age12 H - Age22 H	0.238240038	0.064218976	3.709807517	0.005666309
27	Age12 H - Age32 H	0.442056705	0.059293544	7.455393589	0
28	Age22 H - Age32 H	0.203816667	0.049813793	4.09157093	0.001303719

Table S7. Statistics of ovariole count analysis

	Sum Sq	Df	F values	Pr(>F)
Age	5.426794108	3	1.734958397	0.158954631
Larva_diet	5.366664255	1	5.147204239	0.023734553
Adult_diet	3.679419674	1	3.52895647	0.060921841
Age:Larva_diet	2.782942898	3	0.889713163	0.446304793
Age:Adult_diet	7.227416149	3	2.310621351	0.075530165
Larva_diet:Adult_diet	0.417764673	1	0.400680943	0.527044353
Age:Larva_diet:Adult_diet	1.886405902	3	0.60308825	0.61325716
Residuals	492.1245419	472		

Table S8. Statistics of total number of egg chambers analysis

	Sum Sq	Df	F values	Pr(>F)
Age	32.7745394	3	10.7480422	9.85E-07
Larva_diet	3.35437909	1	3.30009288	0.070260667
Adult_diet	1.32614443	1	1.30468253	0.254258509
Age:Larva_diet	3.05159834	3	1.00073741	0.39280036
Age:Adult_diet	4.57140451	3	1.49914078	0.214819233
Larva_diet:Adult_diet	1.5508089	1	1.5257111	0.217711034
Age:Larva_diet:Adult_diet	4.11664552	3	1.35000768	0.258255327
Residuals	309.000771	304		

Table S9. Statistics of total number of egg chambers Tukey analysis of age

	contrast	estimate	SE	z.ratio	p.value
1	Age10 - Age12	-0.0955729	0.02589544	-3.6907232	0.001279
2	Age10 - Age22	0.0593743	0.02604457	2.27971918	0.10274425
3	Age10 - Age32	0.14889751	0.02614321	5.6954568	7.37E-08
4	Age12 - Age22	0.15494721	0.02595313	5.97027086	1.42E-08
5	Age12 - Age32	0.24447042	0.02605212	9.38389893	3.81E-14
6	Age22 - Age32	0.08952321	0.02620035	3.41687097	0.00354336

Table S10. Statistics of proportion of egg chambers that undergo vitellogenesis analysis

	Sum Sq	Df	F values	Pr(>F)
Age	18.0578967	3	26.7634219	2.18E-15
Larva_diet	3.58789726	1	15.9527562	8.15E-05
Adult_diet	0.01112342	1	0.0494577	0.82415895
Age:Larva_diet	6.27983446	3	9.30727767	6.62E-06
Age:Adult_diet	3.36694766	3	4.99011829	0.00215433
Larva_diet:Adult_diet	0.06114059	1	0.27184751	0.60247475
Age:Larva_diet:Adult_diet	1.66189051	3	2.46307073	0.06256847
Residuals	68.3719322	304		

Table S11. Statistics of proportion of egg chambers that undergo vitellogenesis Tukey analysis for developmental diet x age

	contrast	estimate	SE	t.ratio	p.value
1	Age10 L - Age12 L	-0.4837534	0.10604425	-4.561807	0.00019678
2	Age10 L - Age22 L	0.03944173	0.10604425	0.37193649	0.99995294
3	Age10 L - Age32 L	0.56275149	0.10604425	5.30676089	5.94E-06
4	Age10 L - Age10 H	-0.5481839	0.10604425	-5.1693878	1.17E-05
5	Age10 L - Age12 H	-0.6878537	0.10604425	-6.4864776	9.94E-09
6	Age10 L - Age22 H	0.23236086	0.10604425	2.19116879	0.36000048
7	Age10 L - Age32 H	0.45862326	0.10604425	4.32482896	0.00054355
8	Age12 L - Age22 L	0.52319514	0.10604425	4.93374345	3.63E-05
9	Age12 L - Age32 L	1.04650491	0.10604425	9.86856785	1.11E-12
10	Age12 L - Age10 H	-0.0644305	0.10604425	-0.6075808	0.99876642
11	Age12 L - Age12 H	-0.2041003	0.10604425	-1.9246706	0.53500833
12	Age12 L - Age22 H	0.71611427	0.10604425	6.75297575	2.06E-09
13	Age12 L - Age32 H	0.94237667	0.10604425	8.88663592	1.11E-12
14	Age22 L - Age32 L	0.52330977	0.10604425	4.9348244	3.61E-05
15	Age22 L - Age10 H	-0.5876256	0.10604425	-5.5413242	1.80E-06
16	Age22 L - Age12 H	-0.7272954	0.10604425	-6.8584141	1.09E-09
17	Age22 L - Age22 H	0.19291913	0.10604425	1.81923231	0.60746972
18	Age22 L - Age32 H	0.41918153	0.10604425	3.95289248	0.00241583
19	Age32 L - Age10 H	-1.1109354	0.10604425	-10.476149	1.11E-12
20	Age32 L - Age12 H	-1.2506052	0.10604425	-11.793238	1.04E-12
21	Age32 L - Age22 H	-0.3303906	0.10604425	-3.1155921	0.0416121
22	Age32 L - Age32 H	-0.1041282	0.10604425	-0.9819319	0.97672288
23	Age10 H - Age12 H	-0.1396698	0.10604425	-1.3170899	0.89190859
24	Age10 H - Age22 H	0.78054472	0.10604425	7.36055655	4.93E-11
25	Age10 H - Age32 H	1.00680712	0.10604425	9.49421672	1.11E-12
26	Age12 H - Age22 H	0.92021453	0.10604425	8.6776464	1.12E-12
27	Age12 H - Age32 H	1.14647693	0.10604425	10.8113066	1.09E-12
28	Age22 H - Age32 H	0.2262624	0.10604425	2.13366017	0.39570379

Table S12. Statistics of proportion of egg chambers that undergo vitellogenesis Tukey analysis for adult diet x age

	contrast	estimate	SE	t.ratio	p.value
1	Age10 L - Age12 L	-0.234723	0.10604425	-2.2134442	0.34659296
2	Age10 L - Age22 L	0.84192362	0.10604425	7.93936113	2.22E-12
3	Age10 L - Age32 L	1.19093221	0.10604425	11.230521	1.07E-12
4	Age10 L - Age10 H	0.02194137	0.10604425	0.20690771	0.99999917
5	Age10 L - Age12 H	-0.3667588	0.10604425	-3.4585449	0.01421278
6	Age10 L - Age22 H	4.20E-06	0.10604425	3.96E-05	1
7	Age10 L - Age32 H	0.40056777	0.10604425	3.77736427	0.00466414
8	Age12 L - Age22 L	1.07664666	0.10604425	10.1528053	1.11E-12
9	Age12 L - Age32 L	1.42565525	0.10604425	13.4439652	1.04E-12
10	Age12 L - Age10 H	0.25666441	0.10604425	2.4203519	0.23527282
11	Age12 L - Age12 H	-0.1320358	0.10604425	-1.2451007	0.91762645
12	Age12 L - Age22 H	0.23472724	0.10604425	2.2134838	0.34656934
13	Age12 L - Age32 H	0.63529081	0.10604425	5.99080847	1.64E-07
14	Age22 L - Age32 L	0.34900859	0.10604425	3.29115991	0.02441546
15	Age22 L - Age10 H	-0.8199822	0.10604425	-7.7324534	5.47E-12
16	Age22 L - Age12 H	-1.2086824	0.10604425	-11.397906	1.06E-12
17	Age22 L - Age22 H	-0.8419194	0.10604425	-7.9393215	2.22E-12
18	Age22 L - Age32 H	-0.4413558	0.10604425	-4.1619969	0.00106111
19	Age32 L - Age10 H	-1.1689908	0.10604425	-11.023613	1.08E-12
20	Age32 L - Age12 H	-1.557691	0.10604425	-14.689066	1.04E-12
21	Age32 L - Age22 H	-1.190928	0.10604425	-11.230481	1.07E-12
22	Age32 L - Age32 H	-0.7903644	0.10604425	-7.4531568	2.78E-11
23	Age10 H - Age12 H	-0.3887002	0.10604425	-3.6654526	0.00698063
24	Age10 H - Age22 H	-0.0219372	0.10604425	-0.2068681	0.99999917
25	Age10 H - Age32 H	0.3786264	0.10604425	3.57045656	0.00973018
26	Age12 H - Age22 H	0.36676301	0.10604425	3.45858452	0.01421091
27	Age12 H - Age32 H	0.76732658	0.10604425	7.23590918	1.07E-10
28	Age22 H - Age32 H	0.40056357	0.10604425	3.77732466	0.00466481

Table S13. Statistics of Viable Progeny Count from day 1 adults analysis

	Sum Sq	Df	F values	Pr(>F)
Larva_diet	12.9349316	1	15.28558589	0.000156037
Adult_diet	65.41637194	1	77.30443442	0
Larva_diet:Adult_diet	19.88411763	1	23.49764167	3.91411E-06
Residuals	98.16124006	116		

Table S14. Statistics of Viable Progeny Count from day 1 adults Tukey analysis for developmental diet x adult diet

	contrast	estimate	SE	z.ratio	p.value
1	LL - HL	-0.35882225	0.099758474	-3.596909974	0.001829318
2	LL - LH	-0.791345393	0.097621592	-8.106253746	0
3	LL - HH	-0.539139798	0.098760118	-5.459084171	2.86473E-07
4	HL - LH	-0.432523143	0.094928846	-4.556287776	3.08536E-05
5	HL - HH	-0.180317548	0.09609928	-1.876367308	0.23821306
6	LH - HH	0.252205595	0.093879144	2.686492263	0.036322199