Title: Additive and context-dependent effects of developmental and adult diet on life-history traits and their associations in outbred *Drosophila melanogaster* population

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Data availability:

Life-history data will be made available on Dryad Digital Repository upon acceptance of the manuscript.

Conflict of interest:

The authors declare no conflict of interest.

Abstract

Life-history traits such as body size, reproduction, survival, and stress resistance are fundamental to an organism's fitness and are highly influenced by nutritional environments at different life stages. In this study, we used a full factorial experimental design with isocaloric diets varying in protein-to-carbohydrate ratios during both developmental and adult stages to investigate their effects on key life-history traits in an outbred *Drosophila melanogaster* population. Our results demonstrated significant diet-induced plasticity, with body size predominantly determined by the developmental diet, where flies raised on carbohydrate-rich diets were larger as adults. Fertility, however, was enhanced by protein-rich diets at both developmental and adult stages, emphasizing the role of protein in reproductive success. Lifespan was strongly stage-dependent, with carbohydrate-rich developmental diets extending adult lifespan, while the same diet in adulthood shortened it. Furthermore, starvation resistance was improved by a protein-rich developmental diet, whereas desiccation resistance was enhanced by a carbohydrate-rich adult diet, illustrating distinct stage-specific nutritional effects on stress tolerance. Notably, no interactions were found between developmental and adult diets for any of these traits, suggesting independent and additive effects of nutrition across life stages. Additionally, the relationships between traits, such as body size and fertility or lifespan and reproduction, shifted depending on dietary context, challenging canonical trade-offs and highlighting the plasticity of trait correlations. These findings suggest that the nutritional environment can decouple established life-history trait correlations, contributing to context-dependent evolutionary trajectories. This highlights the need to account for both developmental and adult dietary environments when examining how organisms adapt to changing ecological conditions.

Impact Summary

Our study uncovers how isocaloric diets with varying macronutrient compositions at both developmental and adult stages decouple traditional life-history trait correlations in Drosophila melanogaster, adding new layers of complexity to life-history evolution. Using a large, outbred population and a full factorial design, we show that developmental and adult nutrition independently and additively shape key adult traits, including body size, reproductive output, lifespan, and stress resistance. For instance, a carbohydrate-rich developmental diet increased body size, while protein-rich diets at both stages boosted reproductive output. Notably, carbohydrate-rich adult diets, while reducing lifespan, improved resistance to stressors such as starvation and desiccation.

Since the underlying nutrient-sensing and metabolic pathways are conserved across species, our findings are broadly relevant beyond Drosophila. The ability of nutrition to independently influence traits at different life stages suggests that selection can act flexibly across the life cycle, allowing organisms to adapt more readily to changing ecological conditions.

Crucially, our results uncouple canonical correlations among life-history traits, such as the relationships between body size and fertility, fertility and longevity, and longevity and stress resistance. These associations are not fixed but shift based on dietary context. This highlights the importance of considering both developmental and adult dietary environments in studies of evolutionary adaptation, as these interactions may profoundly influence how organisms respond to ecological change.

Keywords: Canonical trait correlation, Life-history traits, Macronutrients, P:C ratio, Isocaloric diet, Developmental diet, Adult diet

1. Introduction

The nutritional environment directly impacts Darwinian fitness of organisms and, thereby, shapes their ecological and evolutionary trajectories by influencing various life history traits, including body size, reproductive output, longevity, and stress resistance (Flatt, 2020; Martin & Bize, 2018). Macronutrients like proteins, carbohydrates and lipids consumed in food are used to generate ATP, the body's energy currency, which supports and sustains various biological processes. However, the importance of food extends far beyond merely providing energy. Macronutrients also serve as building blocks for protein, nucleotide, and lipid synthesis (Nehme et al., 2023; Prentice, 2005). They contribute to stored reserves like glycogen and lipids for future use (Olsen et al., 2021) and often act as signaling molecules that trigger growth, development, and cognitive functions (Rodriguez et al., 2017), thereby crucially influencing organismal life history and performance.

Thus, not surprisingly, a several studies focused on understanding the relationship between dietary macronutrients and life history traits, distinguishing these effects from caloric content using isocaloric diets with varying macronutrient compositions (Shi et al., 2021; Solon-Biet et al., 2014). Despite extensive research, the precise relationship between dietary macronutrients and life history traits remains unclear. For example, longevity, one of the most well-studied life history traits in this context, has shown diverse responses to varying dietary protein-to-carbohydrate (P:C) ratios in diets. Previous studies have demonstrated that P:C ratios can have negative (Lushchak et al., 2012), positive (Bruce et al., 2013) and complex non-monotonic (Lee, 2015)impacts on longevity. Similarly, while some studies have found that adult body size increases with higher P:C ratios (Rodrigues et al., 2015), others have observed that body size maximizes at intermediate ratios (Lee & Roh, 2010).

One potential reason for the observed discrepancies in organismal performance across isocaloric dietary studies stems from the fact that all organisms undergo a pre-reproductive stage of growth and development before becoming reproductively active, and food is crucial for both developmental and reproductive stages. However, dietary requirements during these two stages may differ significantly (Parks et al., 2020). Most research studies have tested organismal performance by manipulating the diet at either the developmental (Dinh et al., 2022; Savola et al., 2022a) or reproductive stage while keeping the diet constant for the other stage, or by applying the same dietary treatment to both stages (Mudunuri et al., 2024). Consequently, these studies could not explicitly capture the interactive effects of diets available at both stages, leaving it as a confounding factor. This issue arises because it is not feasible to provide absolutely no food at one stage to study the impact of diet at the other stage on adult life history. Moreover, the interactive effects of stage-specific diet can vary across different life history traits, further complicating the interpretation of such experimental data. In the simplest scenario, even if there is no significant interaction between the diets available across life stages, drastically different dietary requirements for developmental and reproductive stages can still generate a non-monotonic relationship. Therefore, the observed non-monotonic diet-induced plasticity profiles of various life history traits could be artifacts of mismatched stage-specific dietary needs, the interactive effects between diets at different life stages, or a combination of both. One way to address this is to use a full factorial experimental design involving stage-specific diet availability, which is rarely employed due to its logistical demands (Poças et al., 2022a; Pullock et al., 2023).

Additionally, investigating multiple traits under diverse dietary environments provides a unique opportunity to explore the associations between various life history traits. These associations arise because life history traits do not exist in isolation; rather, they are all interconnected aspects of the same organism. Consequently, several studies have

demonstrated a canonical negative association, or trade-off, between energy-demanding traits such as reproductive output and longevity or stress resistance (Strilbytska et al., 2024), considering finite energy reserve of an individual. Conversely, body size is typically observed to have a positive association with fertility (Honek, 1993), longevity (Khazaeli et al., 2005), and stress resistance traits (Ballard et al., 2008), as greater size often indicates more energy resources available for allocation to various life history traits and vice versa. Moreover, a positive association between longevity and stress resistance has also been observed (Epel & Lithgow, 2014) as both benefit from greater energy allocation to body maintenance.

These canonical associations are important because they provide insights into the evolutionary strategies organisms use to optimize their fitness and predict eco-evolutionary outcomes. Practically, these insights inform conservation efforts, agricultural practices, and medical research, aiding in improving health and longevity, managing wildlife resources, and optimizing breeding programs for crops and livestock. Given that nutrition can drastically alter the expression of these life history traits, it provides an opportunity to evaluate the associations between these traits under contrasting dietary environments. However, existing data in the literature are not very helpful because most studies focus on one or two traits (Klepsatel et al., 2020; May et al., 2015) or several aspects of a single life history trait (Krittika & Yadav, 2022; Mirth et al., 2019).Comparing life history results across multiple studies is challenging due to variations in experimental details, including the genetic background of the organisms, diet composition, caloric content, and the use of solid versus liquid food (Gillette et al., 2020; Lüersen et al., 2019), all of which can affect the measured trait levels. Therefore, a comprehensive study assessing the performance of key fitness-related traits—encompassing longevity, reproductive output, and stress resistance—under common isocaloric dietary regimes with contrasting macronutrient compositions is warranted.

We addressed the above knowledge gaps using a fully factorial experimental design with isocaloric diets having high and low protein-to-carbohydrate ratios for both developmental and adult stages. We used a large, genetically heterogeneous *Drosophila melanogaster* population as a model system. In each diet regime, we measured five life-history traits: body size, fertility, longevity, and two stress resistance traits—starvation resistance and desiccation resistance. We also compared the associations between these traits across the contrasting dietary regimes. Our results reveal an additive interaction between developmental and adult diets in shaping these five key life history traits, and highlight the importance of stage-specific dietary composition in determining association between them.

2. Materials and Methods:

2.1 Maintenance of the experimental Population

Ancestry of the experimental population used in this study traces back to the large, outbred, laboratory-maintained *Drosophila melanogaster* Baseline Population (BP), whose origin and maintenance details were reported in a previous article (Mudunuri A. et al. 2023). BP population was reared for 30 generations on corn-sugar-yeast medium (CSY) with protein-to-carbohydrate (P:C) ratio of 0.4. Subsequently, from this BP population, five identical large outbred populations, BP_{1-5}, were derived. These five replicate populations were further maintained in parallel and identically for subsequent 10 generations in the same manner as the original BP population prior to the current study. Briefly, they were reared at 25 temperature and 60% relative humidity, under constant light, in a 14-day discrete generation cycle. To initiate a new generation, ~300 eggs were collected per 150 ml fly culturing bottle (FLBT-20) containing 50 ml of CSY medium with P:C ratio of 0.4. The adults were transferred to a plexi-glass cage (25 cm x 20 cm x 15 cm) on the 11th day following egg collection and breading population size was maintained to ~2100 individuals. On the 13th day, these populations were allowed ~14 hours to lay eggs on the CSY medium, maintaining the same diet composition, to start the next generation. For this study, BP_4 population was chosen at random to produce the experimental flies, which were then subjected to contrasting experimental diet regimes.

2.2 Experimental diet regimes

In this study, we took two isocaloric diets with distinct compositions: a carbohydrate-rich diet with a P:C ratio of 0.25 (henceforth referred to as the C diet) and a protein-rich diet with a P:C ratio of 0.7 (henceforth referred to as the P diet). These diets were formulated following

established protocols (Mudunuri et al., 2024). Both of the P and C diets were provided during the larval and adult stages in a full factorial design, resulting in four distinct experimental diet regimes: CC, CP, PC, and PP. The first letter in each pair indicates the larval diet, and the second letter indicates the adult diet. For instance, CP represents a diet regime that begins with a carbohydrate-rich diet during the larval stage, followed by a protein-rich diet during the adult stage. We subjected the experimental flies to these diet regimes and assayed five key life-history traits.

2.3 Generation of the experimental flies

Before conducting each life history assay, to ensure minimizing the effect of larval crowding, we generated the experimental flies by collecting approximately 150 eggs per fly culture bottle from the BP_4 population, each containing 50 ml of appropriate larval diet. The number of bottles was adjusted according to the total number of adult flies needed for each specific assay. The switch from larval to adult diet was implemented at the pupal stage by replacing the developmental diet cup with an appropriate adult diet. This ensured that, upon eclosion from the pupal case, the adults encountered their respective adult diets. All assays were performed on the 12th day after egg collection, ensuring that the adult flies had consumed both the larval and adult diets for a comparable duration.

2.4 Body size measurement

To determine body size across experimental diet regimes, we measured wing length as a proxy in both males and females (Reeve, 1953). This was done by carefully clipping off one wing, chosen randomly from each fly with fine forceps. The clipped wings were mounted on a glass slides using PEG 6000 (SRL, Cat: 25322-68-3) with 30 replicates for each sex in each

regime. The images of mounted wings were captured using a Zeiss Stemi 305 microscope with Axiocam 105 colour camera under 40X magnification. The final measurement of the wing was done by measuring the length between ACV to L3 nodes using ImageJ software, calibrated with a standard scale (Stage micrometer-ESM11). This assay involved the measurement of a total 240 wings (30 replicates × 2 sex × 4 regimes), and was subjected to statistical analysis.

2.5 Fertility assay

In order to estimate the reproductive output across experimental diet regimes, we collected 12th day (post egg collection) mated flies for assay setup. This was done by sorting flies using mild CO_2 and randomly placing 2 males and 2 females in a vial containing the respective adult diet, 30 such replicates were created for each regime. Further, the vials were incubated for 24 hrs under standard laboratory condition for flies to lay eggs. After 24 hrs, the adult flies were discarded and the vials containing eggs were incubated under standard laboratory conditions and the number of eclosed adult offsprings was recorded as fertility of the mating pairs. Fertility per mating pair was calculated and subjected to statistical analysis. This fertility assay involved a total of 480 flies for all four regimes (30 replicates × 2 flies × 2 sex × 4 regimes).

2.6 Longevity assay

For measuring lifespan of flies across four experimental diet regimes, 8 adult males and 8 adult females were sorted using mild CO_2 and 10 such replicates were separated by sex in vials containing ~5ml of respective adult diet and were incubated under standard laboratory conditions. After the experimental setup, each vial was monitored every day once for counting the number of alive flies per vial. Fresh food was provided every third day until all

flies were dead in a vial. The lifespan of flies was estimated as the number of days they lived after the assay setup was done. This setup involved assessing lifespan of 640 flies (10 replicates × 8 flies × 2 sex × 4 regimes) for all four regimes. The average lifespan data of the 8 flies in each vial was calculated and these vial means were subjected for the statistical analysis.

2.7 Desiccation resistance assay

Desiccation resistance across experimental diet regimes were determined by sorting 8 males and 8 females in each vial and 10 such replicated were used. These vials were incubated under standard laboratory conditions. After the experimental setup, each vial was monitored every 2 hrs for counting the number of alive flies per vial. The desiccation resistance of flies was estimated as the number of hours they lived after the assay setup was done. This setup involved measuring the desiccation resistance of 640 flies (10 replicates × 8 flies × 2 sex × 4 regimes) for all four regimes. The average desiccation resistance data of the 8 flies in each vial was calculated and the mean value was subjected for the statistical analysis.

2.8 Starvation resistance assay

To assess starvation resistance the flies were separated into 8 males and 8 females in each replicate vial and 10 such replicates were maintained in a vial containing ~1% agar. These vials were incubated under standard laboratory conditions. After the experimental setup, each vial was monitored every 4 hrs for counting the number of alive flies per vial. Fresh ~1% agar vial was provided every third day until all flies were dead in a vial. The starvation resistance of flies was estimated as the number of hours they lived after the assay setup was done. This setup involved 640 flies (10 replicates × 8 flies × 2 sex × 4 regimes) for all four

regimes. The mean starvation resistance of 8 flies for each vial was subjected for the statistical analysis.

2.9 Statistical analysis:

To determine the impact of composition of larval diet and adult diet on adult traits such as body size, body weight, longevity, starvation and desiccation resistance, data for these assays were subjected to a three-way fixed factor ANOVA with developmental diet (P and C), adult diet (P and C) and sex (Male and Female) as fixed factors. Whereas, fertility assay data was subjected to a two-way factor ANOVA with developmental diet (P and C), and adult diet (P and C) as fixed factors. If main effect or interaction was significant, the post-hoc analysis was performed using Tukey's HSD test. STATISTICA®v7 (StatSoft. Inc., Tulsa, Oklahoma) software was used for all statistical analysis. Additionally, effect size for every trait was computed using Cohen's d to measure the variations between dietary composition of each stages-specific diet. These effect sizes were categorized as small ($0.2 < d < 0.5$), medium ($0.5 < d < 0.8$), or large ($d > 0.8$) according to Cohen's criteria (Cohen, 1988). The visual representations of developmental diet and adult diet were generated using ggplot2 and patchwork package within R version 4.2.1 and RStudio v2022.06.23 (RStudio Team, 2020).

2.10 Trait association index:

To analyze the association between traits in a pair-wise manner for each life stage, we formulated an Association Index (AI). We calculated the mean trait values for both P (protein-rich) and C (carbohydrate-rich) diets at each life stage (developmental and adult). Since the units of measurement and absolute values of the traits were not uniform, we first computed the percentage change in the mean trait value under the P diet compared to the C diet for each trait at each life stage. The AI for two traits was then determined by taking the ratio of the percentage change in trait 1 to the percentage change in trait 2. Mathematically,

$AI_{1,2}$= (%change in mean trait 1 under P diet relative to C diet)/ (%change in mean trait 2 under P diet relative to C diet). By following this approach, we obtained AI values for each pair-wise trait association under both diets and life stages. When two traits change in a similar fashion, the AI is positive; conversely, if one trait increases while the other decreases under similar conditions, the AI is negative. Here, our primary focus was on the nature of the associations, indicated by the sign of the AI index, which would be identical for $AI_{1,2}$ and $AI_{2,1}$.

3. Results

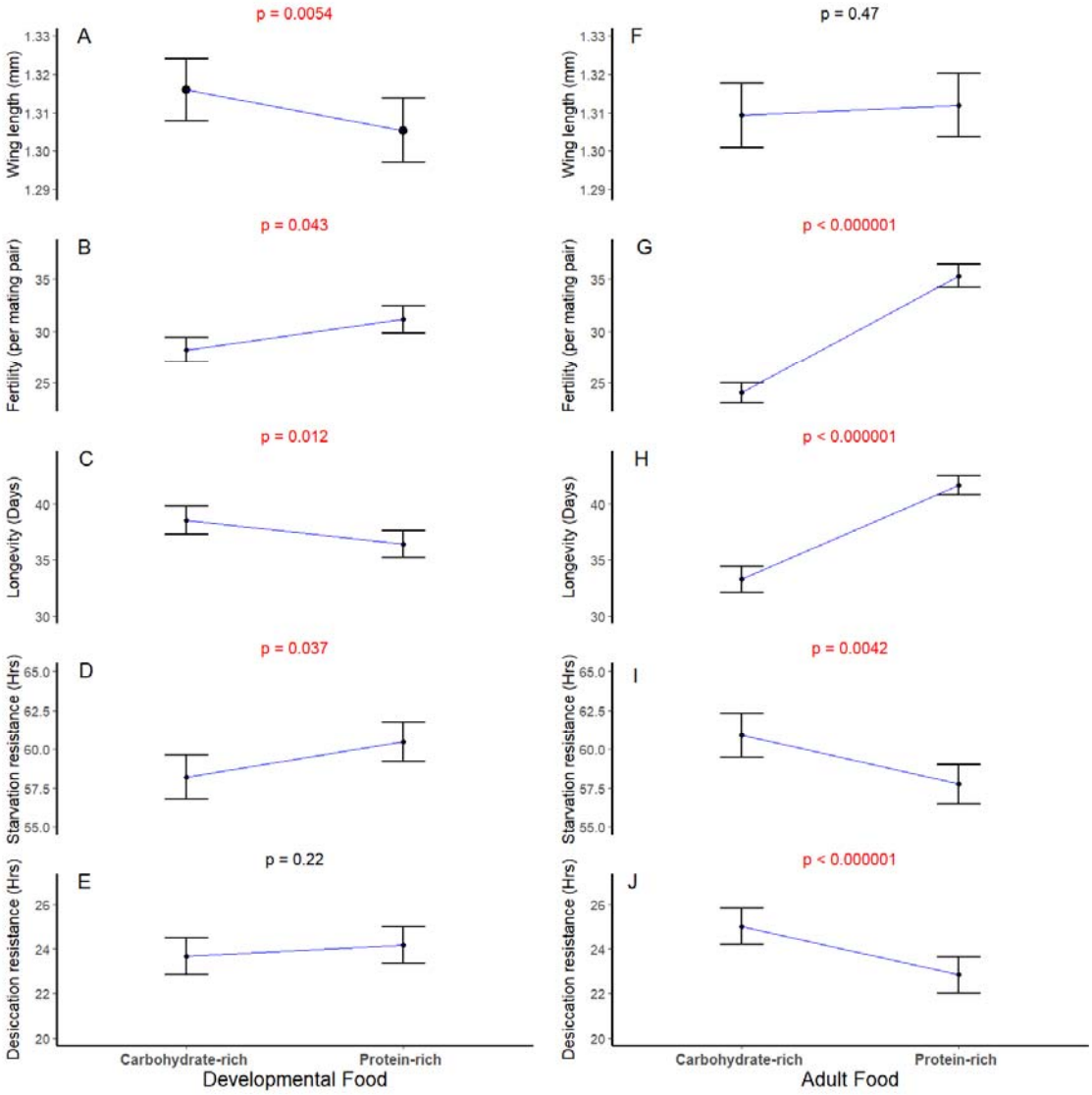

Figure 1: Effect of developmental and adult diet composition on major life-history traits. The graphs depict the impact of carbohydrate-rich and protein-rich during the larval stage (A to E) and adult stage (F to J) on various life-history traits in *Drosophila melanogaster*. Life-history traits measured includes body size, fertility, longevity, starvation resistance and desiccation resistance. The error bar depicts the standard error (± SE) of mean for each trait with significant differences between diets at both larval and adult stage indicated by p-values in red. The blue line connects between the mean of two dietary treatments.

Developmental diet composition primarily affects body size

We found a significant effect of developmental diet on wing length, a proxy for body size (Figure 1A, $F_{1,232} = 7.9$, $p = 0.0054$). Flies reared on a carbohydrate-rich developmental diet had larger wing lengths compared to those reared on a protein-rich developmental diet.

The main effect of adult diet on body size was non-significant (Figure 1F, $F_{1,232} = 0.5$, $p = 0.47$), and there was no significant interaction between developmental diet and adult diet ($F_{1,232} = 0.5$, $p = 0.46$). Wing length remained unaffected by the composition of the adult diet or by its interaction with the developmental diet.

As expected in *D. melanogaster*, females were bigger in size with significantly larger wing lengths than males ($F_{1,232} = 2075.9$, $p < 0.000001$). However, the interactions between developmental diet and sex (Figure S1A, $F_{1,232} = 0.7$, p = 0.41), between sex and adult diet (Figure S1B, $F_{1,232} = < 0.00001$, $p = 1.0$), and the three-way interaction between developmental diet, adult diet, and sex ($F_{1,232} = 1.5$, $p = 0.22$) were not significant. This indicates an absence of sex differences in the impact of stage-specific dietary composition on body size.

Fertility is influenced by both developmental and adult diets

Developmental diet had a significant impact on fertility (Figure 1B, $F_{1,116} = 4.1$, $p = 0.043$), with flies fed a protein-rich developmental diet producing more viable offspring.

A strong significant effect of adult diet on reproductive output was observed (Figure 1G, $F_{1,116} = 61.1$, $p < 0.000001$), with flies fed a protein-rich adult diet producing more offspring than those fed a carbohydrate-rich adult diet. The interaction between developmental diet and

adult diet was not significant ($F_{1,116} = 0.06$, $p = 0.8$), suggesting that the availability of protein-rich food, irrespective of life stage, enhances adult offspring production.

Longevity is affected by developmental and adult diets in opposite ways

The developmental diet significantly influenced the longevity of flies (Figure 1C, $F_{1,72} = 6.5$, $p = 0.012$), with a carbohydrate-rich developmental diet extending adult lifespan. Conversely, the adult diet also had a significant impact on lifespan (Figure 1H, $F_{1,72} = 102.2$, $p < 0.000001$), with flies fed a protein-rich adult diet living longer than those on a carbohydrate-rich adult diet. But interestingly, the two-way interaction between developmental diet and adult diet was not significant ($F_{1,72} = 1.4$, $p = 0.23$).

Males lived longer than females ($F_{1,72} = 151.6$, $p < 0.000001$). The non-significant interaction between developmental diet and sex (Figure S2A, $F_{1,72} = 3.2$, $p = 0.07$) indicates a similar effect of developmental diet on both sexes, while the significant interaction between sex and adult diet (Figure S2B, $F_{1,72} = 4.5$, $p = 0.03$) suggests a sex-biased impact of adult diet composition on longevity. However, the three-way interaction among developmental diet, adult diet, and sex was not significant ($F_{1,72} = 2.8$, $p = 0.09$).

Divergent impacts of developmental and adult diets on starvation resistance

Developmental diet significantly affected starvation resistance in adults (Figure 1D, $F_{1,72} = 4.5$, $p = 0.037$), with flies fed a protein-rich developmental diet showing higher resistance to starvation. Similarly, adult diet had a significant impact on starvation resistance (Figure 1I, $F_{1,72} = 8.7$, $p = 0.004$), with flies fed a carbohydrate-rich adult diet exhibiting higher

resistance compared to those on a protein-rich diet. Interestingly, the interaction between developmental diet and adult diet was not significant ($F_{1,72} = 0.5$, $p = 0.47$).

Females had significantly higher starvation resistance than males (F1,72 = 155.8, p < 0.000001), and this sex difference was not influenced by developmental diet (Figure S3A, developmental diet × sex: $F_{1,72} = 2$, $p = 0.16$), adult diet (Figure S3B, adult diet × sex: $F_{1,72} = 3.8$, $p = 0.054$), or their interaction (developmental diet × adult diet × sex: $F_{1,72} = 3.4$, $p = 0.07$).

Desiccation resistance is primarily affected by adult diet

Developmental diet did not significantly affect desiccation resistance in adults (Figure 1E, F1,72 = 1.5, p = 0.22). In contrast, the adult diet had a significant impact (Figure 1J, F1,72 = 32, p < 0.000001), with flies reared on a carbohydrate-rich adult diet showing higher tolerance to desiccation stress. The interaction between developmental diet and adult diet on desiccation resistance was not significant (F1,72 = 0.01, p = 0.941).

Similar to starvation resistance, females had significantly higher desiccation resistance than males (F1,72 = 614.4, p < 0.000001), and this sex difference was not influenced by developmental diet (Figure S4A, developmental diet × sex: F1,72 = 0.2, p = 0.61), adult diet (Figure S4B, adult diet × sex: F1,72 = 0.02, p = 0.87), or their interaction (developmental diet × adult diet × sex: F1,72 = 0.06, p = 0.8).

Pair-wise life-history trait associations

We found that stage-specific diets impact associations between adult traits (see Table 1 for all pair-wise AI values). Under the developmental diet, body size was negatively associated with fertility, while a positive association was observed under the adult diet. Similarly, the association between fertility and longevity was negative under the developmental diet but positive under the adult diet. The canonical negative associations between fertility and stress

resistance traits (starvation and desiccation resistance) were observed under the adult diet, while they were positively associated under the developmental diet. Other trait associations remained consistent across both diets. Positive associations were observed between body size and longevity, and between the two stress resistance traits under both diets. Interestingly, body size and longevity were negatively associated with the two stress resistance traits under both diets.

Table 1: Association index for pair-wise trait combinations under developmental and adult diets

	Association Index (AI)	
Trait combinations	**Developmental diet**	**Adult diet**
Body size vs Fertility	-9.2	122.6
Body size vs Longevity	4.8	66.2
Body size vs Starvation resistance	-3.5	-13.3
Body size vs Desiccation resistance	-1.7	-22.5
Fertility vs Longevity	-0.5	0.5
Fertility vs Starvation resistance	0.4	-0.1
Fertility vs Desiccation resistance	0.2	-0.2
Longevity vs Starvation resistance	-0.7	-0.2
Longevity vs Desiccation resistance	-0.3	-0.3
Starvation resistance vs Desiccation resistance	0.5	1.7

4. **Discussion**:

Life-history traits are deeply intertwined with organismal response to the environmental cues, including the availability and composition of nutrients at various life stages. In this study, we investigated the effects of dietary composition by altering the relative proportion of proteins and carbohydrates in both developmental and adult stages in *Drosophila melanogaster*. Using a full factorial design, we assessed the effects of these stage-specific dietary manipulations on key life-history traits-body size, fertility, longevity, starvation resistance and desiccation resistance-and evaluated their pair-wise associations. Our results showed significant effect of both developmental and adult diet on these traits, with no significant interactive effects between the two stages. We also found that the pair-wise associations between the life history traits were context specific, depending on the life stage and diet composition.

Body size is a key determinant of organism's fitness, influencing its various aspects such as mating success, lifespan, avoiding predation, and stress resistance(Jungers, 1986; Rose & Mueller, 1993). Our study showed that adult wing length (a proxy for body size) of flies is primarily determined by diet available during their developmental stage, rather than adult diet. Moreover, flies reared on carbohydrate-rich developmental diets had larger wing lengths compared to those fed with protein-rich developmental diets. These findings are consistent with the earlier studies demonstrating body size is predominantly determined by the diet available during juvenile/developmental stage(Nijhout, 2003; Poças et al., 2022). This suggests that developmental stage pays a critical role, with diet fed during this stage having a more pronounced impact on energy reserve and growth, ultimately influencing adult body size. One explanation for this pattern is that larvae reared on carbohydrate-rich diets compensate for protein restriction by consuming more food (Almeida de Carvalho & Mirth, 2017). This overconsumption of high-carbohydrate food likely to increases their fat storage.

Another study on this model system (Enriquez et al., 2022) showed that fat content during larval stage is positively correlated with pupal size and thereby determining the size of adults in *D. melanogaster*. Thus, carbohydrate-rich developmental diet could lead to bigger body size in adults. In contract, another study, where the caloric content of diets was not kept constant, found increased *Drosophila* body size under a low-sugar diet via sex-specific mechanisms (Millington et al., 2022). This opens up opportunities for further mechanistic investigations into diet-induced plasticity in body size. Additionally, we did not find an interaction between developmental and adult diet, indicating that the effect of stage-specific diets was additive. Similarly, our results did not show any interaction between sex and diet, indicating that the impact of these diets at each stage was consistent across both male and females. This contrasts with, a study that investigated various aspects of *Drosophila* adult body size across age under contrasting developmental and adult diet, where they found a significant age-dependent interaction between stage-specific diet with sex for some aspects of adult body size, such as body weight (Poças et al., 2022). This highlights a more nuanced aspect of diet-induced plasticity, which warrants further investigations.

Reproduction is a crucial fitness trait, directly influencing genetic contribution to future generations (Cockburn et al., 2011). Nutrition plays a significant role in reproductive success (Smykal & Raikhel, 2015). In our study, we found that flies fed with a protein-rich developmental diet produced more viable offspring. This aligns with previous research showing that increased dietary protein enhances fertility in *Drosophila melanogaster* (Lee et al., 2008; Schultzhaus & Carney, 2017). Additionally, protein rich diets during the adult stage also significantly boosted fertility. Thus, protein-rich diets irrespective of stages increased number of offspring production compared to carbohydrate-rich diets. Interestingly however, we found no significant interaction between developmental and adult diets. This result is consistent with a previous study on the same model system, demonstrating that

developmental and adult diets impact fertility independently (Ruchitha et al., 2024). The reason behind fertility boost with high-protein diets is likely due to the requirement of dietary amino acids for egg production in (Bowman & Tatar, 2016), as evidenced by protein-rich diets leading to greater number of ovarioles in female ovaries and facilitating vitellogenesis process (Ruchitha et al., 2024). This suggests a complex interplay between stage-specific diets in shaping reproductive success.

Lifespan is a complex trait shaped by various factors, including nutrition, with efficient nutrient utilization often leading to longer survival and greater reproductive success (Lee et al., 2008). Our study highlights the distinct effects of developmental and adult diets on lifespan. Interestingly, we showed that carbohydrate-rich developmental diet is beneficial for adult lifespan, which is in contrast to a previous study showing developmental diet composition has no effect on lifespan. Conversely, other research supports our results, showing that a carbohydrate-rich diet can extend Drosophila lifespan (Lee et al., 2008; Savola et al., 2022). Similarly, a previous study (Tatar et al., 2014) showed that diets with low protein-to-carbohydrate ratios extend lifespan by regulating dILP6 in adult fat body. Interestingly, while a carbohydrate-rich diet extends lifespan when consumed during the larval stage, it shortens lifespan when consumed by adults—a detrimental effect which was noted previously as well (Lushchak et al., 2014). We did not observe any interaction between developmental and adult diets, suggesting their effects on lifespan are additive. However, we did find an interaction between adult diet and sex, indicating that the adult diet impacts male and female lifespans differentially. These results illustrate the intricate relationship between developmental and adult diets in shaping adult lifespan. Further mechanistic investigations on nutrient sensing signalling like insulin/IGF-like signalling (IIS) and its impact on lifespan will help to dissect the nuanced effects of nutrition on lifespan.

Stress resistance traits, such as starvation resistance and desiccation resistance in adults modulated by several physiological processes where nutrition plays a pivotal role (Brown et al., 2019). Our study demonstrates a significant effect of developmental diet on adult starvation resistance. Flies raised on protein-rich developmental diets showed greater resistance to starvation, a result that appears contrasting with earlier studies suggesting that low-nutrient developmental diets, achieved through food dilutions, increase fat stores and starvation resistance (Rehman & Varghese, 2021). However, this discrepancy can be explained by the fact that the previous study did not use isocaloric diets. Consistent with our findings, other studies support the beneficial effect of protein-rich developmental diet on stress resistance (Andersen et al., 2010). Adequate protein intake during developmental stage promotes muscle development and efficient nutrient storage, aiding resistance to starvation (Gillette et al., 2021). Interestingly, the trend reverses in adult diets, with carbohydrate-rich adult diets conferring greater starvation resistance compared to protein-rich diets, aligning with studies that show decreased starvation resistance with increasing protein-to-carbohydrate ratios (Gerofotis et al., 2019; Lee & Jang, 2014; Simmons & Bradley, 1997). These findings suggest that while protein is critical during larval development, excess carbohydrate during adult stage increases the adult fat storage (Asiimwe et al., 2023), enhancing starvation resistance. Importantly, we did not observe any interaction between larval and adult diets, nor between sex and diets, indicating that the effects of stage-specific diets on starvation resistance are consistent across both males and females.

For desiccation resistance, another stress resistance trait, we did not observe a significant impact of developmental diet composition. However, the adult diet had a significant effect on desiccation resistance. The differential impact of diet during these two life stages likely stems from the nature of desiccation stress, which is more acute than starvation stress.

Consequently, it is not surprising that the immediate adult diet, rather than the developmental diet, played a more prominent role in influencing this trait. Although a previous study reported that a protein-rich diet during the larval stage enhanced desiccation resistance in adults (Andersen et al., 2010), the discrepancy between our results and theirs may be due to the use of isocaloric diets in our study, unlike in the previous one. Flies fed a carbohydrate-rich diet during their adult stage exhibited greater resistance to desiccation stress compared to those fed a protein-rich diet. Carbohydrate-rich diets are known to increase glycogen and lipid reserves in flies (Djawdan et al., 1998). Glycogen, in particular, plays a key role in enhancing desiccation resistance by helping to retain water (Archer et al., 2007; GIBBS et al., 2004). This explains the increased desiccation tolerance observed in our study. Additionally, we found no significant two-way or three-way interaction between developmental diet, adult diet, and sex, indicating that these effects were additive and consistent across both sexes. Future research focusing on glycogen content quantification and assays on younger flies could provide deeper insights into the stage-specific dietary effects on desiccation resistance.

In short, our results demonstrate an impressive diet-induced plasticity in five key life history traits in *Drosophila* that have direct implications on their Darwinian fitness. In this section we highlight four general observations and their broader implications. First, there is no universally best diet regime. The effects of dietary composition on traits vary by life stages and the specific trait in question, emphasizing the importance of timing and context in dietary interventions. This also brings out the complexity of ecological interactions and the trade-offs organisms must navigate to maximize fitness. Such trade-offs will shape how populations adapt to environmental changes, such as habitat loss or climate change, as they influence the availability and quality of food resources. Second, our study highlights that both developmental and adult diets significantly affect adult traits. Despite undergoing complete

metamorphosis, where larval tissues are largely replaced, developmental nutrition still plays a vital role in shaping adult outcomes. Previous research suggests that larval fat cells, acting as nutrient reservoirs during (Aguila et al., 2007; Andersen et al., 2010), may drive this effect, but further research is needed to fully understand the mechanisms involved. The influence of early nutrition emphasizes its long-term impact on fitness and adaptability across diverse ecological contexts, with broader implications for evolutionary biology. Third, our full factorial experimental design allowed us to examine potential interactions between developmental and adult diets. We found no significant interactions, indicating that the nutritional environment during development influences adult traits independently of the adult diet. This finding warrants further investigation into the mechanisms by which stage-specific diets shape life history traits. Finally, we observed that stage-specific diets significantly influence the associations between adult traits, demonstrating that trait correlations are highly context-dependent. For example, body size was negatively associated with fertility under the developmental diet, while a positive correlation between these two traits was observed in the context of adult diets. A similar pattern was found for the relationship between fertility and longevity, which shifted from negative under the developmental diet to positive under the adult diet. Recent study has found that the decoupling of canonically known fertility and lifespan trade-off is influenced by the developmental diet (Collins et al., 2023). These findings contrast with the canonical view that body size and fertility are usually positively correlated (Sarikaya et al., 2019) and that reproductive output typically comes at the cost of longevity (Hunt et al., 2004; Partridge et al., 2005). Interestingly, the relationships between fertility and stress resistance traits (starvation and desiccation resistance) also shifted between the diets. While the adult diet reinforced the expected negative correlation between fertility and stress resistance (Wang et al., 2001), the developmental diet showed positive associations. This suggests that nutritional environments early in life may alter the allocation

of resources in a way that allows for simultaneous benefits to reproductive output and stress resistance, an idea supported by some studies on plastic responses to early-life nutrition (Boggs, 2009). Consistent across both diets were the positive associations between body size and longevity, echoing earlier findings that larger body size often leads to greater resource storage and extended lifespan (Chown & Gaston, 2010). Similarly, a consistent positive association was observed between starvation and desiccation resistance, indicating common coping mechanisms for these environmental stresses. On the other hand, the observed negative correlations between body size and longevity with the two stress resistance traits under both diets suggest an evolutionary trade-off, where investment in size and lifespan may come at the cost of reduced acute stress tolerance. This pattern highlights the complexity of resource allocation across life history traits and aligns with past research on the energetic costs of maintaining large body size and longer lifespan (Rose & Mueller, 1993). In short, trait associations are not fixed but are highly sensitive to the nutritional environment, particularly in the context of life history trade-offs. Understanding how these correlations shift across developmental and adult diets is crucial for predicting evolutionary trajectories, especially in changing environments.

Conclusion:

Our study highlights the importance of both developmental and adult nutrition on key life-history traits in *Drosophila melanogaster*. By altering the protein-to-carbohydrate ratio while maintaining isocaloric diets during the developmental and adult stages, we observed significant changes in body size, fertility, longevity, starvation resistance, and desiccation resistance. Notably, our results suggest that carbohydrate-rich developmental diet enhanced body size, whereas protein-rich adult diets, while beneficial to increase longevity, proved

detrimental under stress, such as starvation and desiccation. Furthermore, we found that developmental and adult diet can independently modulate adult traits, and no dietary regime optimally benefits for all the traits simultaneously. Additionally, our study highlights how trait correlations, such as those between body size, fertility, longevity and stress resistance, are context dependent and shaped by nutritional environment. These results emphasize the complexity of dietary impacts on life-history traits and their associations, offering valuable insights into how stage-specific dietary manipulations can influence evolutionary and ecological outcomes of organisms.

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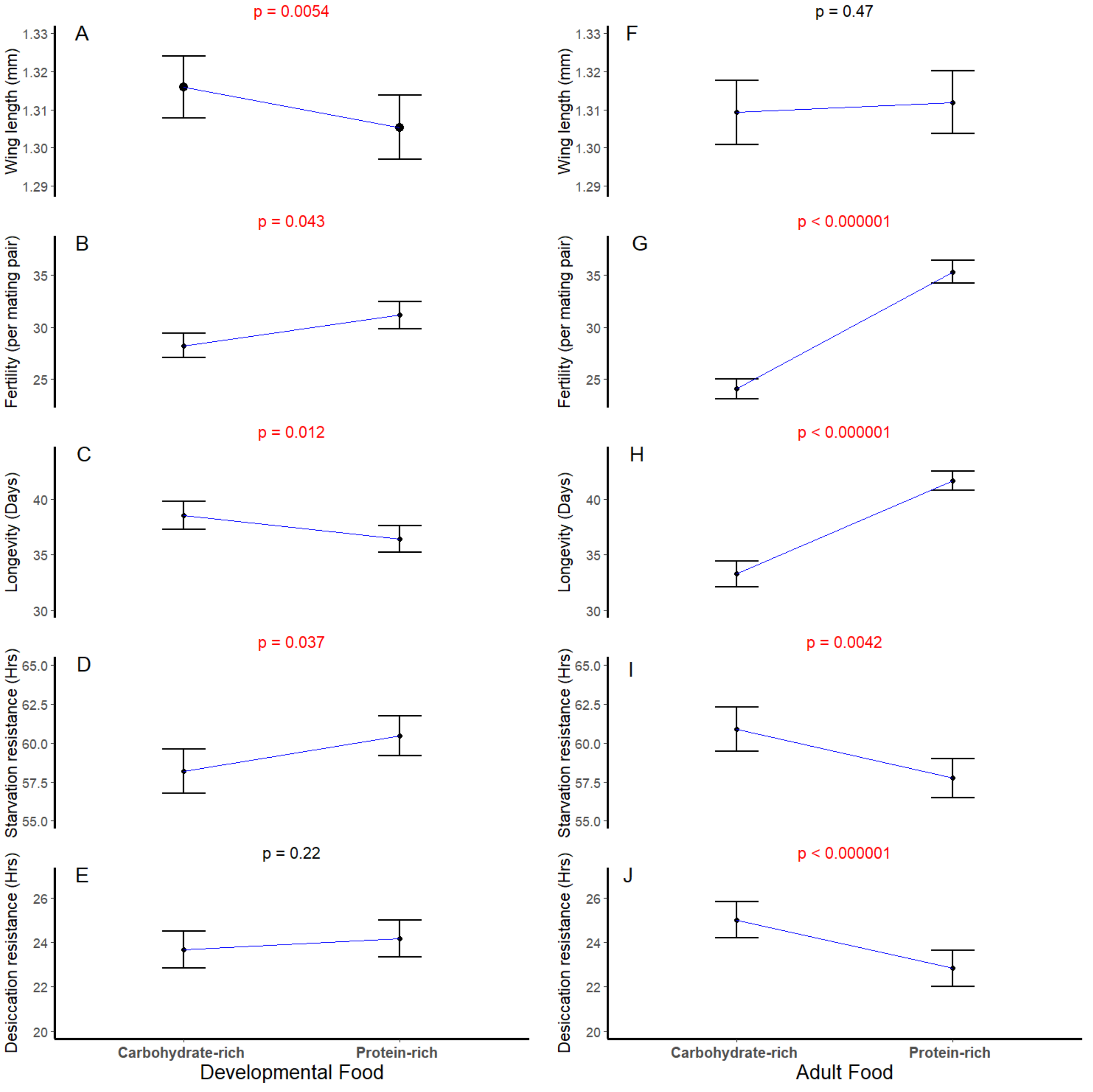
p = 0.0054
A
Wing length (mm)
1.33
1.32
1.31
1.30
1.29
p = 0.47
F
p = 0.043
B
Fertility (per mating pair)
35
30
25
p < 0.000001
G
p = 0.012
C
Longevity (Days)
40
35
30
p < 0.000001
H
p = 0.037
D
Starvation resistance (Hrs)
65.0
62.5
60.0
57.5
55.0
p = 0.0042
I
p = 0.22
E
Desiccation resistance (Hrs)
26
24
22
20
p < 0.000001
J
Carbohydrate-rich
Protein-rich
Developmental Food
Adult Food