© 2018. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2018) 221, jeb181115. doi:10.1242/jeb.181115

RESEARCH ARTICLE

Comparing the impacts of macronutrients on life-history traits in larval and adult *Drosophila melanogaster*: the use of nutritional geometry and chemically defined diets

Taehwan Jang and Kwang Pum Lee*

ABSTRACT

Protein and carbohydrate are the two major macronutrients that exert profound influences over fitness in many organisms, including *Drosophila melanogaster*. Our understanding of how these macronutrients shape the components of fitness in *D. melanogaster* has been greatly enhanced by the use of nutritional geometry, but most nutritional geometric analyses on this species have been conducted using semi-synthetic diets that are not chemically well defined. Here, we combined the use of nutritional geometry and chemically defined diets to compare the patterns of larval and adult life-history traits expressed across 34 diets systematically varying in protein:carbohydrate (P:C) ratio and in protein plus carbohydrate (P+C) concentration. The shape of the response surfaces constructed for all larval and adult traits differed significantly from one another, with the nutritional optima being identified at P:C 1:4 for lifespan (P+C 120 g l^{-1}), 1:2 for egg-to-adult viability (120 g l^{-1}), 1:1 for female body mass at adult eclosion (240 g l^{-1}) and lifetime fecundity (360 g l^{-1}), 2:1 for larval developmental rate (60 g l^{-1}) and 8:1 for egg production rate (120 g l^{-1}). Such divergence in nutritional optima among life-history traits indicates that *D. melanogaster* confined to a single diet cannot maximize the expression of these traits simultaneously and thus may face a life-history trade-off. Our data provide the most comprehensive and nutritionally explicit analysis of the impacts of macronutrients on life-history traits in *D. melanogaster* and support the emerging notion that the fundamental trade-offs among life-history traits are mediated by macronutrients.

KEY WORDS: Carbohydrate, Development, Fecundity, Life-history trade-off, Lifespan, Protein

INTRODUCTION

Macronutrients have major impacts on nearly all components of organismal fitness, with protein and carbohydrate being the two most influential macronutrients that have been the subject of intensive investigation in recent literature on nutritional ecology (Simpson and Raubenheimer, 2012). The effects of these macronutrients are extremely complicated because they operate not only simultaneously but also interactively (Simpson et al., 2015). For example, adding a specific macronutrient to the diet not only increases its own content but also alters the relative balance between the macronutrients in the diet, an aspect that has substantial consequences for fitness in its own right. Further adding to the complexity is that the effects of protein and carbohydrate are not always linear. Our current understanding of the role played by macronutrients in shaping the fitness of an organism has been greatly advanced by the use of nutritional geometry (NG), a multi-dimensional state–space modeling approach which offers an integrative framework for describing how the components of fitness are quantitatively and qualitatively associated with these interacting macronutrients (Simpson and Raubenheimer, 2012). Numerous studies have adopted NG as a standard methodology for disentangling the complex effects of macronutrients (protein, carbohydrate, lipids) on physiological, morphological and life-history traits in diverse organisms ranging from slime mold to primates (see literature cited in Simpson and Raubenheimer, 2012).

NG has proved particularly instrumental in exploring how macronutrients mediate the trade-off between lifespan and reproduction, the two most fundamental components of adult fitness (Simpson et al., 2015). The first study that used NG to identify the nutritional mediation of the lifespan–reproduction relationship was conducted on *Drosophila melanogaster*, a key model organism for aging research, by Lee et al. (2008). In that study, the lifespan and reproductive outcome of female *D. melanogaster* were recorded from 1008 mated individuals confined to 28 semi-synthetic diets varying in protein:carbohydrate (P:C) ratio and in total caloric density. Lee et al. (2008) found that lifespan and egg laying rate were maximized at different P:C ratios in female *D. melanogaster*, with the former peaking at P:C 1:16 and the latter at P:C 1:2. The most important implication that has arisen from these results is that the classical life-history trade-offs between lifespan and reproduction may arise because these two traits have completely different macronutrient requirements for maximizing their expression, rather than because they compete for a finite pool of resources as has been traditionally assumed (Lee et al., 2008; Boggs, 2009; Flatt, 2011; Simpson et al., 2015). Qualitatively similar patterns of diverging nutritional optima between lifespan and egg laying rate have been corroborated in other organisms, including *Bactrocera* fruit flies (Fanson et al., 2009; Fanson and Taylor, 2012), crickets (Maklakov et al., 2008) and even mice (Solon-Biet et al., 2014, 2015), suggesting that the physiological and molecular mechanisms underlying the nutritional control of the trade-off between these two fitness components may be highly conserved across taxonomic boundaries.

In recent years, there have been an increasing number of studies that have employed NG to examine the effects of dietary protein and carbohydrate on traits expressed during larval developmental stages in holometabolous insects (Simpson et al., 2004; Sentinella et al., 2013; Rodrigues et al., 2015; Matavelli et al., 2015; Silva-Soares et al., 2017; Gray et al., 2018; Young et al., 2018). By comparing response surfaces plotted for various larval traits expressed on 28

Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Republic of Korea.

*Author for correspondence (kwanglee@snu.ac.kr)

K.P.L., 0000-0001-9770-0078

Received 16 March 2018; Accepted 28 August 2018

Journal of Experimental Biology

Journal of Experimental Biology (2018) 221, jeb181115. doi:10.1242/jeb.181115

semi-synthetic diets varying in P:C ratio and in total caloric content, for example, Rodrigues et al. (2015) found that the optimal P:C ratio for the rate of larval development (P:C 1:2) was lower than that for body size and survival (P:C 1.5:1) in *D. melanogaster*. Sentinella et al. (2013) also used NG to report that an increase in dietary protein content decreased the percentage of larval survival but increased the expression of male secondary sexual traits (i.e. head length) in *Telostylinus angusticollis*. These results indicate that macronutrients may also mediate the trade-offs between larval life-history traits.

Whilst the majority of NG studies in insects have focused on the expression of fitness traits within a single life stage (larvae or adult), there is a relative paucity of published research examining how macronutrients influence life-history traits expressed throughout entire life stages in a single species. *Drosophila melanogaster* is one of the few insect species in which adult and larval fitness traits have been mapped onto response surfaces using NG, but the traits expressed during the adult and larval stage were measured in separate studies conducted by Lee et al. (2008) and Rodrigues et al. (2015). Despite being prepared based on a similar protocol, 28 semi-synthetic diets used for assaying adult traits in Lee et al. (2008) and those used for assaying larval traits in Rodrigues et al. (2015) differed not only in their physical state (liquid versus solid form) but also in the type of yeast derivatives used as the protein source, thus making any direct comparisons between the data obtained from these studies difficult. It is important to note that yeast types or strains differ considerably in their nutritional quality and thus in their effects on lifespan and reproduction in *D. melanogaster* (Bass et al., 2007).

In order to compare the life-history responses of adult and larval *D. melanogaster* to nutrition directly and explicitly, it is necessary to conduct a study in which adult and larval *D. melanogaster* are exposed to the same experimental diets precisely manipulated for their macronutrient composition. As yeasts are poorly defined ingredients, the development of chemically defined diets containing known amounts of nutrients has been a critical requirement in studies of aging and lifespan in *D. melanogaster* (Piper, 2017). In recent years, an increasing number of studies have used various types of chemically defined or holidic diets to investigate how protein and carbohydrate influence lifespan and reproduction in adult *D. melanogaster* (Troen et al., 2007; Lee and Micchelli, 2013; Piper et al., 2014; Lee, 2015; Jensen et al., 2015), but no full-scale nutritional geometric analyses on larval traits have yet been conducted using a large number of chemically defined diets in this species.

In this study, we combined the use of NG and chemically defined diets to examine the impacts of dietary protein and carbohydrate on a suite of life-history traits expressed during different life stages in *D. melanogaster*. We created 34 chemically defined diets by systematically manipulating P:C ratios and total protein plus carbohydrate (P+C) concentrations. Firstly, we raised larvae from egg to adult emergence on these 34 diets and measured the following larval traits expressed on each diet: egg-to-adult viability, developmental rate and body mass at adult eclosion. Secondly, we allocated newly emerged adult flies to 34 diets and recorded the following adult fitness traits: lifespan, early life egg production rate and lifetime fecundity. The patterns of these larval and adult traits expressed across a range of dietary protein and carbohydrate were visually represented as the response surfaces, which enabled us to locate the nutritional optima for these measured traits. In the current study, we are particularly interested in addressing whether and how the nutritional optima differ among life-history traits expressed within and across ontogenetic stages in this model organism.

MATERIALS AND METHODS

Fly culture

Wild-type Canton-S strain *D. melanogaster* flies were cultured on a standard *Drosophila* rearing diet (90.6 g dextrose, 68 g dry yeast, 42.8 g cornmeal, 6.5 g agar, 4.5 ml propionic acid and 1 g Nipagin in 1 l distilled water) at 25°C under a 12 h:12 h light:dark photoregime. To minimize any parental and grandparental effects associated with different larval rearing density, all experimental flies were raised at a consistent density of ca. 200–250 larvae in 150 ml fly bottles for three continuous generations before the commencement of this study. The protocol for achieving a consistent larval density across rearing bottles is based on the method described by Clancy and Kennington (2001).

Experimental diets

Following the protocol described in Lee et al. (2013), we prepared a total of 34 chemically defined diets that varied in P:C ratio and in P+C concentration. The P:C ratios used were 0:1, 1:16, 1:8, 1:4, 1:2, 1:1, 2:1, 4:1 and 8:1. For each of these nine ratios, there were three or four P+C concentrations: 60, 120, 240 and 360 g l^{-1}. The exact protein and carbohydrate compositions of the 34 experimental diets are outlined in the 2D protein–carbohydrate plane in Fig. 1. As protein and carbohydrate yield similar amounts of calories per unit mass (4 kcal g^{-1}), we assumed those diets with the same P+C concentration to be isocaloric. Sodium caseinate (Sigma C8654) and sucrose (Sigma S9378) were used as the source of protein and

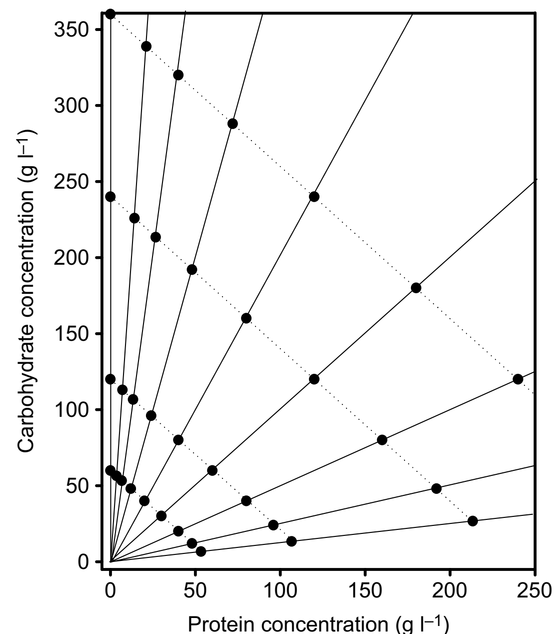

Fig. 1. Graphic description of the 34 chemically defined diets used in this study. Each point describes the amount of protein and carbohydrate present in 1 liter of agar-gelled medium in each of 34 experimental diets that varied in protein:carbohydrate (P:C) ratio and in protein plus carbohydrate (P+C) concentration (see Table S1). Nutritional rails (solid lines) radiating from the origin represent nine P:C ratios (from left to right: 0:1, 1:16, 1:8, 1:4, 1:2, 1:1, 2:1, 4:1 and 8:1). Along each rail, there are three or four different diets, representing different P+C concentrations (extending from the origin: 60, 120, 240 and 360 g l^{-1}). Across different nutritional rails, diets that have the same P+C concentration are connected by isocaloric lines (dotted lines).

Journal of Experimental Biology

carbohydrate, respectively, in these diets. All diets were prepared in 100 ml lots, containing fixed quantities of dietary lipids (30 mg cholesterol and 400 mg lecithin), salts (71 mg KH_2PO_4, 373 mg K_2HPO_4, 62 mg $MgSO_4$ and 100 mg $NaHCO_3$), nucleic acids (57 mg uridine and 64 mg inosine), vitamins (0.2 mg thiamine, 1 mg riboflavin, 1.2 mg nicotinic acid, 1.67 mg calcium pantothenate, 0.25 mg pyridoxine, 0.02 mg biotin and 0.3 mg folic acid), solidifying agent (2 g agar) and preservatives (0.1 g Nipagin and 0.3 ml propionic acid) (Sang, 1956). Diets were produced by homogeneously dissolving all pre-weighed ingredients except vitamins and preservatives in sterile distilled water. The suspension was then autoclaved at 121°C for 10–15 min. When the autoclaved suspension had cooled to <50°C, vitamins and preservatives were added and the final volume of the medium was adjusted to 100 ml by adding distilled water. After vigorous stirring, the agar-gelled medium was dispensed into 20 ml fly vials in either 4 ml (adult trait assays) or 7 ml (larval trait assays) aliquots, stabilized at room temperature for 4 h, and stored at 4°C until use. Fresh diets were made every 2 weeks and diets that were stored for longer than 15 days were discarded. The diets used for assaying larval traits (egg-to-adult viability, developmental rate and body mass at eclosion – see below) were prepared a day before the experiment.

Experiment 1: larval life-history traits

In this study, we measured three larval life-history traits linked to fitness in *D. melanogaster*: egg-to-adult viability, larval developmental rate and body mass at eclosion. To obtain a large number of freshly laid eggs, ca. 1000 freshly emerged male and female adults were released into a plastic cage (21 cm×41 cm×21 cm) supplied with an oviposition substrate (4% agar, 10% molasses seeded with live yeast paste in a 90 mm diameter Petri dish) and water source (150 ml bottle filled with distilled water and capped with water-soaked cotton). After 2 days of habituation, flies received a fresh oviposition substrate and were allowed to lay eggs for 4 h. Eggs laid on the substrate were washed with 1× phosphate-buffered saline (PBS) and the resulting egg suspension was filtered through a nylon mesh (70 µm) to collect eggs. Collected eggs were rinsed with PBS and then transferred to a strip of overhead projector (OHP) film (8 mm×24 mm) using a fine brush. We ensured that similar numbers of eggs (ca. 90–110) were assigned to each film strip. Eggs placed on each film strip were photographed using a Canon EOS 600D digital camera (Canon Inc., Tokyo, Japan) before they were randomly allocated to each 20 ml fly vial containing 7 ml of one of 34 test diets. The exact number of eggs seeded into each vial was later counted from the photographed images of the eggs. There were six replicate vials per diet treatment, resulting in a total of 204 vials being used in this assay. All experimental procedures for assaying larval traits were conducted at 25°C under a 12 h:12 h light:dark photoregime. The protocol for assaying each larval trait is described below.

Egg-to-adult viability

For each replicate vial, egg-to-adult viability was determined as the percentage of eggs that successfully developed into adults. For each diet treatment, egg-to-adult viability was recorded from six replicate vials.

Developmental rate

Developmental rate was calculated as the inverse of the time (h) taken from egg to pupariation for individual flies assigned to three replicate vials per diet treatment. The pupariation time of individual flies was recorded by counting the number of newly emerged puparia attached to the wall of the vials every 3 h.

Body mass at eclosion

For each diet treatment, newly eclosed flies were collected every 3 h, pooled across six replicate vials, and killed by freezing at −20°C. Fly carcasses were sexed by inspecting the sexcomb and then randomly divided into 3–10 replicates of five flies per diet treatment per sex. The unequal number of replicates per diet treatment was due to high larval mortality on extremely low P:C diets (P:C 1:16 and 1:8). Each replicate was dried in an oven set at 65°C for 48 h and weighed to the nearest 1 µg using a BM-22 analytical balance (A & D Co. Ltd, Tokyo, Japan). The body mass of individual flies was calculated by dividing the mass of each replicate by five.

Experiment 2: adult life-history traits

Three adult life-history traits were also quantified in this experiment: lifespan, early-life egg production rate and the index of female lifetime fecundity. These traits were measured using several thousand flies raised on the standard *Drosophila* rearing diet under the same rearing conditions applied for maintaining the baseline fly culture as described above. Newly emerged adult flies of both sexes were collected and allowed 48 h to mate in 150 ml fly bottles supplied with the standard rearing diet (ca. 100–150 flies per bottle). Fully mated flies were sexed under mild CO_2 anesthesia and subjected to the adult trait assays at 25°C under a 12 h:12 h light:dark photoregime following the protocol described below. In order to avoid any undesirable effects of dehydration on adult traits (Ja et al., 2009), we regularly moistened the foam plugs of the fly vials with distilled water.

Lifespan

For logistical reasons, the lifespan assay was conducted using only female *D. melanogaster*. For each diet treatment, ca.100–120 mated female flies were randomly distributed over four 20 ml fly vials containing 4 ml of one of 34 test diets (25–30 flies per replicate vial). The lifespan of individual female flies was recorded daily by counting dead flies from replicate vials until all flies had died. Throughout the lifespan assay, flies were transferred to fresh vials every 2 days.

Early-life egg production rate

Fully mated, 2-day-old male and female flies were randomly paired to produce 12 replicate male–female pairs per diet treatment. Each pair was housed in a 20 ml fly vial provided with 4 ml of one of 34 test diets and allowed to habituate to this environment for 5 days before the first egg count. For each replicate, the number of eggs laid over 24 h was counted for five successive days, with flies being transferred to fresh vials daily. Early-life egg production rate was calculated as the total number of eggs produced over five successive days divided by five.

Lifetime fecundity index

In addition to early-life egg production rate, we measured an index that represents the lifetime or realized fecundity of individual female flies. For each diet treatment, we generated 15 male–female pairs by randomly pairing 2-day-old male and female flies. Each pair was then housed in a 20 ml fly vial containing 4 ml of one of 34 test diets. In each of the first 4 weeks of their adult life, fly pairs were allowed to feed *ad libitum* on chemically defined diets for six successive days before they were transferred to fly vials containing the standard rearing diet (7 ml supplied in each fly vial) at 8, 15, 22

Journal of Experimental Biology (2018) 221, jeb181115. doi:10.1242/jeb.181115

and 29 days post-eclosion. Flies were allowed to lay eggs on the standard rearing diet for 1 day and then returned to their assigned chemically defined diets. This whole cycle was repeated for four consecutive weeks. The vials in which eggs were laid were maintained at 25°C until eggs developed into adults and the number of adult offspring that emerged was counted. For each pair, the index of lifetime fecundity was calculated as the total number of adult offspring produced at 8, 15, 22 and 29 days post-eclosion.

To validate that the index we used in this study is a reliable surrogate measure of lifetime reproductive success in *D. melanogaster*, we performed Pearson's product-moment correlation between the actual and proxy measures of lifetime fecundity that were recorded from 20 male–female fly pairs maintained on the standard rearing diet. To determine the lifetime reproductive output of each pair, we transferred flies to fresh vials every 1 or 2 days and counted the number of offspring that emerged from these vials until the female fly in each pair had died. In this assay, the index of lifetime fecundity was calculated as described above. We found a very strong positive correlation between the index and the total number of offspring produced over a lifetime (ρ=0.977, P<0.001; see Fig. S1), thus supporting the validity of the index we used for representing lifetime fecundity.

Statistical analysis

Thin plate spline response surfaces were plotted to illustrate how adult and larval life-history traits were expressed across the 2D protein–carbohydrate plane using the fields package in R v 2.5.1 (http://www.R-project.org). A major advantage of using this non-parametric thin plate spline technique is that it allows a realistic description of the patterns of our focal traits by not constraining the shape of the response surfaces (Lee et al., 2008). To further assess the detailed nature of the effects of protein and carbohydrate on surface traits, we used parametric, second-order polynomial multiple regressions with the linear (P and C), quadratic (P^2 and C^2) and cross-product (P×C) of dietary protein and carbohydrate concentration as the explanatory variables (Lande and Arnold, 1983). As well as visually comparing the surfaces, we used partial F-tests to test whether the shape differed significantly between two response surfaces (e.g. lifespan versus egg production rate) and also took a sequential model building approach to determine to what extent the shape difference between two surfaces was attributed to linear, quadratic and cross-product effects (Chenoweth and Blows, 2005). Before conducting these pair-wise comparisons, each surface trait was standardized using z-transformation to remove any scaling differences between the traits. Apart from thin plate splines, all statistical analyses were performed using SAS v 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Experiment 1: larval life-history traits

Egg-to-adult viability

Polynomial multiple regression analysis revealed a significant negative quadratic effect of protein on egg-to-adult viability, indicating that this measure of larval survivorship was associated with protein concentration in a convex manner (Fig. 2A). The peak for egg-to-adult viability was found at P:C 1:2 and P+C 120 g l^{-1} (nutrient coordinate: P 40 g l^{-1}, C 80 g l^{-1}). Egg-to-adult viability was generally maintained at a high level (>60%) as long as the P:C ratio of the diet was higher than 1:4, but dropped precipitously as the P:C ratio fell below 1:4 (Fig. 2A). It is worth noting that no larvae reached the puparial stage when diets were completely devoid of protein (P:C 0:1). When the P:C ratio was higher than 1:4, egg-to-adult viability decreased from 80% to 50% as the P+C concentration increased from 120 to 360 g l^{-1}.

Developmental rate

There was a significant negative quadratic effect of protein on larval developmental rate (Table 1), suggesting a convex association between protein concentration and larval developmental rate (Fig. 2B). The rate of larval development was maximized (mean time to reach pupariation: 136 h) at P:C 2:1 and P+C 60 g l^{-1} (P 40 g l^{-1}, C 20 g l^{-1}) and declined as protein concentration in the diet deviated from 40 g l^{-1} (Fig. 2B). Larval developmental rate decreased significantly as carbohydrate concentration increased from 20 to 338.8 g l^{-1}.

Body mass at eclosion

Negative quadratic gradients were found to be significant for both protein and carbohydrate in the regression model fitted for the body mass of newly eclosed female *D. melanogaster* (Table 1). The shape of the response surface plotted for female body mass was thus convex (Fig. 2C), with the peak being located at P:C 1:1 and P+C 240 g l^{-1} (P 120 g l^{-1}, C 120 g l^{-1}). The overall shape of the response surfaces for male and female body mass differed significantly (partial F-test: $F_{5,545}$=9.71, P<0.001). Compared with female body mass, male body mass at eclosion was maximized in a more protein-biased (P:C 4:1) and nutritionally diluted (P+C 120 g l^{-1}) region in the protein–carbohydrate plane (P 96 g l^{-1}, C 24 g l^{-1}) (see Fig. S2).

Experiment 2: adult life-history traits

Lifespan

There were significant negative quadratic effects of both dietary protein and carbohydrate on female lifespan (Table 1). As such, the shape of the response surface plotted for female lifespan was convex (Fig. 2D). Female lifespan was maximized (67 days) at P:C 1:4 and P+C 240 g l^{-1} (P 48 g l^{-1}, C 192 g l^{-1}), but was shortened as both protein and carbohydrate concentration in the diet either increased or decreased from their optimal concentration. Regardless of the P+C concentration, lifespan was shortest at P:C 8:1 (14.8–22.8 days). A bell-shaped relationship between female lifespan and carbohydrate concentration was evident when protein concentration ranged between 20 and 80 g l^{-1} (Fig. 2D).

Early-life egg production rate

Egg production rate showed a significant negative quadratic effect of protein (Table 1), suggesting a convex association between egg production rate and protein concentration in the diet (Fig. 2E). The peak for this trait was identified at P:C 8:1 and P+C 120 g l^{-1} (P 106.7 g l^{-1}, C 13.3 g l^{-1}). Egg production rate decreased as protein concentration deviated from the optimal protein concentration of 106.7 g l^{-1} (Fig. 2E). When protein concentration ranged between 80 and 160 g l^{-1}, egg production rate decreased significantly as carbohydrate concentration increased from 13.3 to 240 g l^{-1}.

Lifetime fecundity index

The lifetime fecundity index was associated with protein concentration in a convex manner (Fig. 2F), as indicated by a significant negative quadratic gradient for protein (Table 1). The lifetime fecundity index was maximized at P:C 1:1 and P+C 360 g l^{-1} (P 180 g l^{-1}, C 180 g l^{-1}) and declined as protein concentration departed from the optimal protein concentration of 180 g l^{-1} (Fig. 2F).

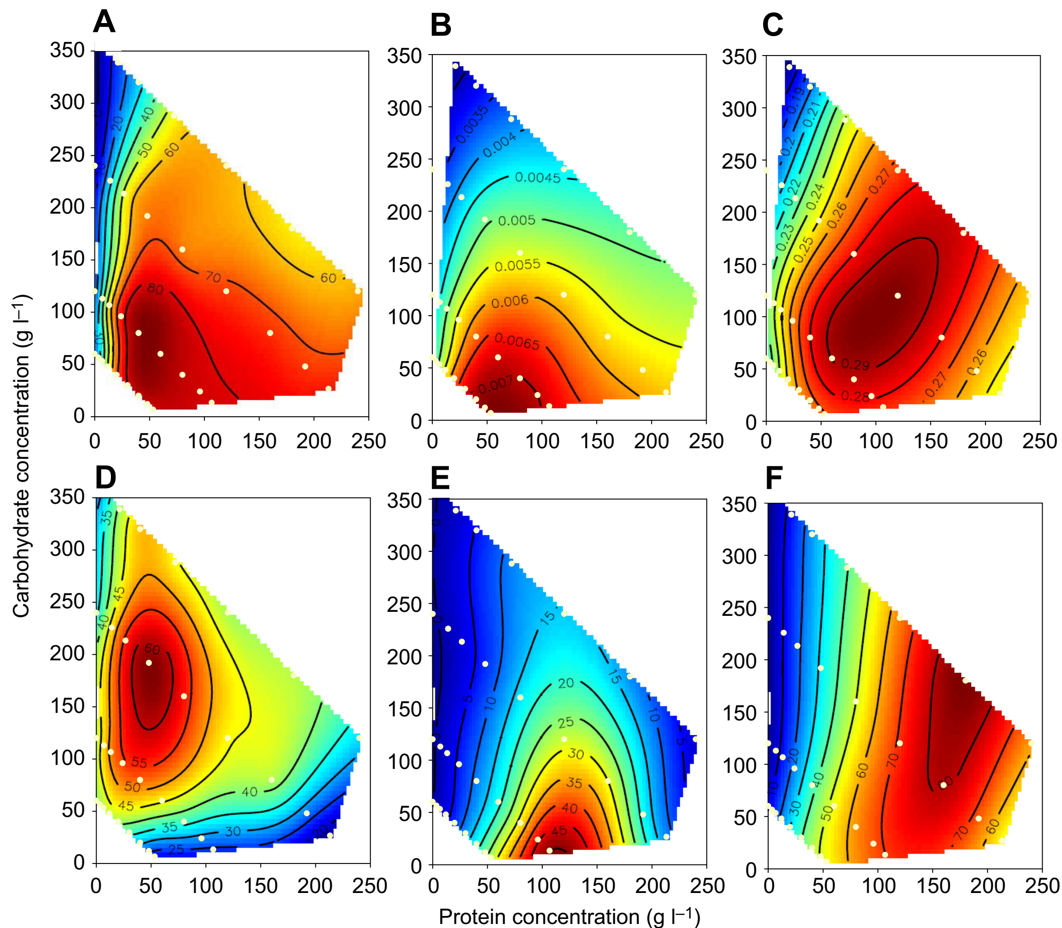

Fig. 2. Thin plate spline response surfaces illustrating the patterns of life-history traits expressed across 34 chemically defined diets. (A) Egg-to-adult viability, (B) larval developmental rate, (C) body mass at adult eclosion (female), (D) adult lifespan (female), (E) egg production rate and (F) the index of lifetime fecundity projected over 34 diets (points) that varied in P:C ratio and P+C concentration (mean values for these traits are summarized in Table S1). The larval and adult traits are shown in the upper (A–C) and lower panels (D–F), respectively. For each response surface, the regions where the traits were expressed at the highest and lowest level are represented by dark red and blue, respectively. Contours are indicated by solid lines and the values are written along the contours.

Comparison of response surfaces

Pair-wise comparisons revealed that the shape of the response surfaces plotted for any of two larval and adult traits differed significantly from one another (partial *F*-test: all $P<0.001$). Full details of the linear, quadratic and cross-product effects contributing to the overall shape difference between two response surfaces are summarized in Table 2.

DISCUSSION

In this study, we combined the use of NG and chemically defined diets to examine the impact of dietary protein and carbohydrate on various life-history traits expressed at larval and adult stages in *D. melanogaster*. Previous studies have repeatedly demonstrated that lifespan and reproduction exhibit a completely different response to dietary protein and carbohydrate in many female insects, with the optimal P:C for reproduction being higher than that for lifespan (Lee et al., 2008; Maklakov et al., 2008; Fanson et al., 2009; Jensen et al., 2015). Consistent with these previous results, we found that the rate of egg production during early adulthood was maximized at a substantially higher P:C ratio (P:C 8:1) than that which supported maximum lifespan (P:C 1:4) in female *D. melanogaster*, reflecting that egg production requires substantial protein investment (Wheeler, 1996). Such divergence in nutritional optima between these two important components of adult fitness suggests that *D. melanogaster* confined to a single diet cannot maximize both lifespan and reproduction at the same time. This finding reinforces the emerging paradigm that the fundamental trade-off between lifespan and reproduction in organisms arises not necessarily because these traits are competing over limited internal resources but because they have different macronutrient requirements for maximal performance (Lee et al., 2008; Boggs, 2009; Flatt, 2011; Simpson et al., 2015). In a manner also similar to previous studies (Lee et al., 2008; Fanson et al., 2009), our data showed that the P:C ratio that maximized the index of lifetime fecundity (P:C 1:1) was intermediate between the ratios that maximized early-life egg production rate (P:C 8:1) and lifespan (P:C 1:4), leading us to predict that the maximal fitness of *D. melanogaster* can be achieved by nutritionally optimizing the trade-off between lifespan and egg production rate.

Numerous studies have documented that the lifespan of *D. melanogaster* and other species was maximized at the lowest P:C ratio of the experimental diets used and decreased progressively as protein content in the diet increased (Lee et al., 2008; Fanson et al., 2009; Dussutour and Simpson, 2012; Jensen et al., 2015; Solon-Biet et al., 2014). In marked contrast to these previous results, our data revealed that the lifespan of *D. melanogaster* was

Table 1. Results of the second-order polynomial multiple regressions on life-history traits expressed across 34 chemically defined diets

	Linear gradients		Quadratic gradients		Cross-product gradients
Response trait	P	C	P^2	C^2	P×C
Larval traits					
Egg-to-adult viability					
Gradient±s.e.	6.97E−01±8.81E−02	−1.20E−01±7.11E−02	−2.88E−03±3.38E−04	−9.62E−05±1.78E−04	2.54E−04±3.77E−04
t_{172}	7.91	−1.68	−8.52	−0.54	0.67
P	<0.001	0.094	<0.001	0.590	0.502
Developmental rate					
Gradient±s.e.	2.35E−05±1.09E−06	−1.8E−05±8.46E−07	−1.21E−07±4.40 E−09	1.56E−08±2.45E−09	3.34E−08±5.17E−09
t_{3922}	21.6	−21.58	−27.48	6.36	6.46
P	<0.001	<0.001	<0.001	<0.001	<0.001
Body mass*					
Gradient±s.e.	1.03E−03±9.36E−05	1.21E−04±7.75E−05	−4.78E−06±3.64E−07	−1.28E−06±2.10E−07	1.66E−06±3.91E−07
t_{271}	10.96	1.56	−13.13	−6.13	4.25
P	<0.001	0.120	<0.001	<0.001	<0.001
Adult traits					
Lifespan*					
Gradient±s.e.	4.49E−02±1.74E−02	3.09E−01±1.34E−02	−6.05E−04±6.62E−05	−8.38E−04±3.39E−05	1.15E−04±6.74E−05
t_{3354}	2.57	23.02	−9.14	−24.71	1.71
P	0.010	<0.001	<0.001	<0.001	0.088
Egg production rate					
Gradient±s.e.	5.77E−01±2.26E−02	−9.80E−02±1.73E−02	−2.20E−03±8.58E−05	2.16E−04±4.39E−05	−4.83E−04±8.96E−05
t_{393}	25.53	−5.65	−25.64	4.92	−5.4
P	<0.001	<0.001	<0.001	<0.001	<0.001
Lifetime fecundity					
Gradient±s.e.	7.92E−01±7.22E−02	−1.94E−01±5.44E−02	−2.90E−03±2.80E−04	3.08E−04±1.37E−04	9.63E−04±2.82E−04
t_{467}	10.97	−3.57	−10.37	2.25	3.41
P	<0.001	<0.001	<0.001	0.025	<0.001

Linear, quadratic and cross-product gradients fitted for dietary protein (P) and carbohydrate (C) concentration are summarized for each trait.
*Measured from females only.

associated with dietary protein content in a convex manner. As clearly illustrated by the bell shape of its response surface, female lifespan was maximized at an optimal protein concentration of 48 g l^{-1} and shortened as protein content in the diet either increased or decreased from this optimum. The physiological mechanisms underlying the life-shortening effect of protein overconsumption remain elusive, but may include toxic effects of nitrogenous breakdown products, mitochondrial generation of radical oxygen species, altered nutrient signaling pathways and reduced immune responses (Sanz et al., 2004; Kapahi et al., 2004; Mirzaei et al., 2014; Le Couteur et al., 2016; Simpson et al., 2017). While much focus has been given to the extension of lifespan by protein restriction, our data clearly show that the restriction of protein intake below a certain threshold shortens lifespan through causing protein starvation (Nakagawa et al., 2012).

Table 2. Pairwise comparisons between the response surfaces plotted for life-history traits

	Effects		
Comparisons	Linear	Quadratic	Cross-product
Egg-to-adult viability			
vs developmental rate	21.45***	2.52ns	1.12ns
vs body mass	1.69ns	14.62***	4.62*
vs lifespan	79.88***	30.97***	0.02ns
vs egg production rate	2.25ns	24.65***	10.88**
vs lifetime fecundity	20.29***	0.79ns	1.46ns
Developmental rate			
vs body mass	53.62***	43.39***	3.26ns
vs lifespan	1097.50***	394.18***	11.32***
vs egg production rate	86.43***	33.08***	37.82***
vs lifetime fecundity	232.79***	0.86ns	0.19ns
Body mass			
vs lifespan	65.93***	32.95***	7.96**
vs egg production rate	0.20ns	79.32***	46.19***
vs lifetime fecundity	14.78***	28.35***	1.59ns
Lifespan			
vs egg production rate	133.10***	197.19***	13.32***
vs lifetime fecundity	181.80***	88.47***	4.34*
Egg production rate			
vs lifetime fecundity	15.02***	28.69***	34.03***

F-ratios generated from partial *F*-tests are summarized for linear, quadratic and cross-product effects of dietary protein and carbohydrate concentration on the shape difference between two given response surfaces. Asterisks indicate significance: *0.01<*P*<0.5; **0.001<*P*<0.01; ****P*<0.001. ns, not significant.

The role of dietary carbohydrate in influencing lifespan has been relatively overlooked compared with that of dietary protein. In this study, we found a clear bell-shaped relationship between dietary carbohydrate content and lifespan in *D. melanogaster*, with lifespan decreasing as carbohydrate concentration in the diet either increased or decreased from an optimal concentration of 192 g l^{-1}. The negative effects of excessive carbohydrate consumption on lifespan and survivorship have been previously described in many insects, including other *D. melanogaster* (Skorupa et al., 2008; Bruce et al., 2013; Lee, 2015), male crickets (Maklakov et al., 2008) and caterpillars (Raubenheimer et al., 2005). It is possible that shortened lifespan in flies fed *ad libitum* on high-carbohydrate diets is due to health deterioration associated with obesity-related metabolic disorder (Musselman et al., 2011), but the exact mechanism underlying the association between excessive carbohydrate intake and shortened lifespan still remains to be elucidated.

In this study, we only focused on exploring the effect of dietary protein and carbohydrate on female reproductive performance (i.e. early-life egg production rate and lifetime fecundity), but recent studies have indicated that macronutrients can also have significant consequences for male reproductive performance in

D. melanogaster (Fricke et al., 2008; Jensen et al., 2015; Morimoto and Wigby, 2016). More interestingly, it has been shown that measures of male reproductive success are maximized in completely different regions in the protein–carbohydrate plane compared with those for female reproductive success in this species (Jensen et al., 2015), indicating that males and females have different nutritional optima for their reproductive success (Maklakov et al., 2008). Further studies are thus warranted to demonstrate how various male reproductive traits are expressed over the same 34 chemically defined diets that were used in the current study. This will certainly provide a more complete picture of the role of dietary protein and carbohydrate in influencing the reproductive success of *D. melanogaster*.

As expected, fly larvae suffered high mortality, retarded development and reduced body size at maturity when raised on diets with extremely low P:C ratios (P:C=0:1, 1:16 and 1:8). The deficiency of protein, a limiting nutrient for growth and survival in immature insects, is likely to be mainly responsible for such poor larval performance on low P:C diets, but the possibility that excessive carbohydrate intake might have also played some role cannot be ruled out (Raubenheimer et al., 2005). Apart from its direct effect on larval fitness, the nutritional quality of the diet consumed by the larvae of holometabolous insects can exert an indirect influence on adult fitness through affecting traits correlating with adult fitness, such as body size and energy reserves (Runagall-McNaull et al., 2015; May et al., 2015). Given the well-established positive association between body size and fecundity in insects (Honěk, 1993), it can therefore be predicted that small adults raised on larval diets with extremely low P:C ratios will produce fewer offspring. A potentially interesting question to be addressed is whether and how flies can buffer the negative effects of protein shortage experienced during their larval stage through adjusting adult behavior and physiology.

There are two preceding studies, one conducted by Rodrigues et al. (2015) and the other by Gray et al. (2018), that used NG to describe the impact of dietary protein and carbohydrate on a suite of larval life-history traits in *D. melanogaster*. Consistent with these previous studies, we found that the nutritional optima for the measured larval traits diverged significantly from one another, indicating the existence of nutrient-mediated life-history trade-offs among these larval traits. However, the results obtained from the present study were qualitatively different from those previously reported by Rodrigues et al. (2015) and Gray et al. (2018) in many aspects. For example, the rate of larval development was found to be maximized at a slightly carbohydrate-biased P:C ratio of 1:2 in Rodrigues et al. (2015) but at a protein-biased P:C ratio of 2:1 in the present study. Furthermore, Gray et al. (2018) reported that the measure of larval survivorship (i.e. egg-to-adult viability) was maintained at a high level (>80%) for *D. melanogaster* raised on diets with extremely low P:C ratios (1:4–1:16). In marked contrast to the results of Gray et al. (2018), the results of this study showed that *D. melanogaster* larvae suffered high mortality when exposed to these low P:C diets during larval development. Such discrepancies between the results of these studies may be due to differences in laboratory fly stock, experimental diets or fly gut microbiota. Whatever the cause, the results obtained from the present and previous studies highlight the need to establish a standardized experimental protocol for investigating the effects of macronutrients on life-history traits in *D. melanogaster* (Bass et al., 2007).

In this study, the response surfaces for life-history traits were constructed based on the concentration of protein and carbohydrate present in each of 34 test diets. This was inevitable because we did not quantify the actual amount of protein and carbohydrate consumed by *D. melanogaster* individuals. As many insects including *D. melanogaster* exhibit a compensatory increase in food intake in response to dietary dilution (Carvalho et al., 2005; Lee et al., 2008; Fanson et al., 2012), one may argue that the concentration-based response surfaces displayed in this study are not the most accurate description of how macronutrient intake is associated with the phenotypic expression of life-history traits in *D. melanogaster*. However, it has been repeatedly documented that compensatory feeding is incomplete in *D. melanogaster* (Carvalho et al., 2005; Lee et al., 2008; Fanson et al., 2012), leading us to assume that *D. melanogaster* will consume a lower amount of macronutrients on more diluted diets. Based on this fact, it is reasonable for us to consider that, although not perfect, the response surfaces plotted based on macronutrient concentrations in this study reliably reflect those based on the actual macronutrient consumption.

In conclusion, this study has comprehensively demonstrated how the nutritional optima for the key components of organismal fitness diverge within and across life stages in *D. melanogaster*, thus providing evidence for nutrient-mediated life-history trade-offs. In this study, the occurrence of nutrient-mediated trade-offs among life-history traits was inferred by visually inspecting whether the nutritional optima for these traits are located in different regions in the response surfaces. Although this approach provides a valid starting point for identifying the potential trade-offs, we acknowledge that such inference based on the visual inspection involves some degree of subjectivity. We hope that a novel statistical approach proposed by Rapkin et al. (2018) will enable us to analyze the existence and strength of nutrient-mediated life-history trade-offs more objectively and quantitatively. Our results indicate that the fitness of *D. melanogaster* individuals is critically dependent upon their ability to achieve an adequate balance of macronutrients from the environment, leading us to postulate an evolutionary hypothesis that natural selection will favor those individuals that can maximize their fitness by adopting optimally foraging and oviposition strategies (Simpson et al., 2004; Lee et al., 2008; Rodrigues et al., 2015; Lihoreau et al., 2016). To the best of our knowledge, this is the first study that has directly compared the responses of larval and adult life-history traits to dietary protein and carbohydrate in *D. melanogaster* using chemically defined diets. We believe that our data reported in this study will provide a useful base for future research on the nutritional biology of this important model organism.

Acknowledgements
We thank the anonymous reviewers for their valuable inputs on an earlier version of the manuscript.

Competing interests
We declare no competing or financial interests.

Author contributions
Conceptualization: T.J., K.P.L.; Methodology: T.J., K.P.L.; Software: K.P.L.; Validation: T.J., K.P.L.; Formal analysis: T.J., K.P.L.; Investigation: T.J., K.P.L.; Resources: K.P.L.; Data curation: K.P.L.; Writing - original draft: T.J., K.P.L.; Writing - review & editing: K.P.L.; Visualization: T.J., K.P.L.; Supervision: K.P.L.; Project administration: K.P.L.; Funding acquisition: K.P.L.

Funding
This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (grant no. NRF-2017R1A2B4004151) and by Seoul National University.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.181115.supplemental

References

Bass, T. M., Grandison, R. C., Wong, R., Martinez, P., Partridge, L. and Piper, M. D. W. (2007). Optimization of dietary restriction protocols in *Drosophila*. *J. Gerontol. A Biol. Sci. Med. Sci.* **62**, 1071-1081.

Boggs, C. L. (2009). Understanding insect life histories and senescence through a resource allocation lens. *Funct. Ecol.* **23**, 27-37.

Bruce, K. D., Hoxha, S., Carvalho, G. B., Yamada, R., Wang, H.-D., Karayan, P., He, S., Brummel, T., Kapahi, P. and Ja, W. W. (2013). High carbohydrate-low protein consumption maximizes *Drosophila* lifespan. *Exp. Gerontol.* **48**, 1129-1135.

Carvalho, G. B., Kapahi, P. and Benzer, S. (2005). Compensatory ingestion upon dietary restriction in *Drosophila melanogaster*. *Nat. Methods* **2**, 813-815.

Chenoweth, S. F. and Blows, M. W. (2005). Contrasting mutual sexual selection on homologous signal traits in *Drosophila serrata*. *Evolution* **165**, 281-289.

Clancy, D. J. and Kennington, W. J. (2001). A simple method to achieve consistent larval density in bottle cultures. *Dros. Info. Service* **84**, 168-169.

Dussutour, A. and Simpson, S. J. (2012). Ant workers die young and colonies collapse when fed a high-protein diet. *Proc. R. Soc. B Biol. Sci.* **279**, 2402-2408.

Fanson, B. G. and Taylor, P. W. (2012). Protein:carbohydrate ratios explain life span patterns found in Queensland fruit fly on diets varying in yeast:sugar ratios. *Age* **34**, 1361-1368.

Fanson, B. G., Weldon, C. W., Pérez-Staples, D., Simpson, S. J. and Taylor, P. W. (2009). Nutrients, not caloric restriction, extend lifespan in Queensland fruit flies (*Bactrocera tryoni*). *Aging Cell* **8**, 514-523.

Fanson, B. G., Yap, S. and Taylor, P. W. (2012). Geometry of compensatory feeding and water consumption in *Drosophila melanogaster*. *J. Exp. Biol.* **215**, 766-773.

Flatt, T. (2011). Survival costs of reproduction in *Drosophila*. *Exp. Gerontol.* **46**, 369-375.

Fricke, C., Bretman, A. and Chapman, T. (2008). Adult male nutrition and reproductive success in *Drosophila melanogaster*. *Evolution* **62**, 3170-3177.

Gray, L. J., Simpson, S. J. and Polak, M. (2018). Fruit flies may face a nutrient-dependent life-history trade-off between secondary sexual trait quality, survival and developmental rate. *J. Insect Physiol.* **104**, 60-70.

Honěk, A. (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* **66**, 483-492.

Ja, W. W., Carvalho, G. B., Zid, B. M., Mak, E. M., Brummel, T. and Benzer, S. (2009). Water- and nutrient-dependent effects of dietary restriction on *Drosophila* lifespan. *Proc. Nat. Acad. Sci. USA* **106**, 18633-18637.

Jensen, K., McClure, C., Priest, N. K. and Hunt, J. (2015). Sex-specific effects of protein and carbohydrate intake on reproduction but not lifespan in *Drosophila melanogaster*. *Aging Cell* **14**, 605-615.

Kapahi, P., Zid, B. M., Harper, T., Koslover, D., Sapin, V. and Benzer, S. (2004). Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. *Curr. Biol.* **14**, 885-890.

Lande, R. and Arnold, S. J. (1983). The measurement of selection on correlated characters. *Evolution* **37**, 1210-1226.

Le Couteur, D. G., Solon-Biet, S., Cogger, V. C., Mitchell, S. J., Senior, A., de Cabo, R., Raubenheimer, D. and Simpson, S. J. (2016). The impact of low-protein high-carbohydrate diets on aging and lifespan. *Cell. Mol. Life Sci.* **73**, 1237-1252.

Lee, K. P. (2015). Dietary protein: carbohydrate balance is a critical modulator of lifespan and reproduction in *Drosophila melanogaster*: a test using a chemically defined diet. *J. Insect Physiol.* **75**, 12-19.

Lee, K. P., Simpson, S. J., Clissold, F. J., Brooks, R., Ballard, J. W. O., Taylor, P. W., Soran, N. and Raubenheimer, D. (2008). Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. *Proc. Nat. Acad. Sci. USA* **105**, 2498-2503.

Lee, K. P., Kim, J.-S. and Min, K.-J. (2013). Sexual dimorphism in nutrient intake and lifespan is mediated by mating in *Drosophila melanogaster*. *Anim. Behav.* **86**, 987-992.

Lee, W.-C. and Micchelli, C. A. (2013). Development and characterization of a chemically defined food for *Drosophila*. *PLoS ONE* **8**, e67308.

Lihoreau, M., Poissonnier, L. A., Isabel, G. and Dussutour, A. (2016). *Drosophila* females trade off good nutrition with high-quality oviposition sites when choosing foods. *J. Exp. Biol.* **219**, 2514-2524.

Maklakov, A. A., Simpson, S. J., Zajitschek, F., Hall, M. D., Dessmann, J., Clissold, F. J., Raubenheimer, D., Bonduriansky, R. and Brooks, R. C. (2008). Sex-specific fitness effects of nutrient intake on reproduction and lifespan. *Curr. Biol.* **18**, 1062-1066.

Matavelli, C., Carvalho, M. J. A., Martins, N. E. and Mirth, C. K. (2015). Differences in larval nutritional requirements and female oviposition preference reflect the order of fruit colonization of *Zaprionus indianus* and *Drosophila simulans*. *J. Insect Physiol.* **82**, 66-74.

May, C. M., Doroszuk, A. and Zwaan, B. J. (2015). The effect of developmental nutrition on life span and fecundity depends on the adult reproductive environment in *Drosophila melanogaster*. *Ecol. Evol.* **5**, 1156-1168.

Mirzaei, H., Suarez, J. A. and Longo, V. D. (2014). Protein and amino acid restriction, aging and disease: from yeast to humans. *Trends Endocrinol. Metab.* **25**, 558-566.

Morimoto, J. and Wigby, S. (2016). Differential effects of male nutrient balance on pre-and post-copulatory traits, and consequences for female reproduction in *Drosophila melanogaster*. *Sci. Rep.* **6**, 27673.

Musselman, L. P., Fink, J. L., Narzinski, K., Ramachandran, P. V., Hathiramani, S. S., Cagan, R. L. and Baranski, T. J. (2011). A high-sugar diet produces obesity and insulin resistance in wild-type *Drosophila*. *Dis. Model. Mech.* **4**, 842-849.

Nakagawa, S., Lagisz, M., Hector, K. L. and Spencer, H. G. (2012). Comparative and meta-analytic insights into life extension via dietary restriction. *Aging Cell* **11**, 401-409.

Piper, M. D. W. (2017). Using artificial diets to understand the nutritional physiology of *Drosophila melanogaster*. *Curr. Opin. Insect Sci.* **23**, 104-111.

Piper, M. D. W., Blanc, E., Leitão-Gonçalves, R., Yang, M., He, X., Linford, N. J., Hoddinott, M. P., Hopfen, C., Soultoukis, G. A., Niemeyer, C. et al. (2014). A holidic medium for *Drosophila melanogaster*. *Nat. Methods* **11**, 100-105.

Rapkin, J., Jensen, K., Archer, C. R., House, C. M., Sakaluk, S. K., del Castillo, E. and Hunt, J. (2018). The geometry of nutrient space-based life-history trade-offs: sex-specific effects of macronutrient intake on the trade-off between encapsulation ability and reproductive effect in decorated crickets. *Am. Nat.* **191**, 452-474.

Raubenheimer, D., Lee, K. P. and Simpson, S. J. (2005). Does Bertrand's rule apply to macronutrients? *Proc. R. Soc. B* **272**, 2429-2434.

Rodrigues, M. A., Martins, N. E., Balancé, L. F., Broom, L. N., Dias, A. J., Fernandes, A. S. D., Rodrigues, F., Sucena, É. and Mirth, C. K. (2015). *Drosophila melanogaster* larvae make nutritional choices that minimize developmental time. *J. Insect Physiol.* **81**, 69-80.

Runagall-McNaull, A., Bonduriansky, R. and Crean, A. J. (2015). Dietary protein and lifespan across the metamorphic boundary: protein-restricted larvae develop into short-lived adults. *Sci. Rep.* **5**, 11783.

Sang, J. H. (1956). The quantitative nutritional requirements of *Drosophila melanogaster*. *J. Exp. Biol.* **33**, 45-72.

Sanz, A., Caro, P. and Barja, G. (2004). Protein restriction without strong caloric restriction decreases mitochondrial oxygen radical production and oxidative DNA damage in rat liver. *J. Bioenerg. Biomembr.* **36**, 545-552.

Sentinella, A. T., Crean, A. J. and Bonduriansky, R. (2013). Dietary protein mediates a trade-off between larval survival and the development of male secondary sexual traits. *Funct. Ecol.* **27**, 1134-1144.

Silva-Soares, N. F., Nogueira-Alves, A., Beldade, P. and Mirth, C. K. (2017). Adaptation to new nutritional environments: larval performance, foraging decisions, and adult oviposition choices in *Drosophila suzukii*. *BMC Ecol.* **17**, 21.

Simpson, S. J. and Raubenheimer, D. (2012). *The Nature of Nutrition: A Unifying Framework from Animal Adaptation to Human Obesity*. Princeton, NJ: Princeton University Press.

Simpson, S. J., Sibly, R. M., Lee, K. P., Behmer, S. T. and Raubenheimer, D. (2004). Optimal foraging when regulating intake of multiple nutrients. *Anim. Behav.* **68**, 1299-1311.

Simpson, S. J., Le Couteur, D. G. and Raubenheimer, D. (2015). Putting the balance back in diet. *Cell* **161**, 18-23.

Simpson, S. J., Le Couteur, D. G., Raubenheimer, D., Solon-Biet, S. M., Cooney, G. J., Cogger, V. C. and Fontana, L. (2017). Dietary protein, aging and nutritional geometry. *Ageing Res. Rev.* **39**, 78-86.

Skorupa, D. A., Dervisefendic, A., Zwiener, J. and Pletcher, S. D. (2008). Dietary composition specifies consumption, obesity, and lifespan in *Drosophila melanogaster*. *Aging Cell* **7**, 478-490.

Solon-Biet, S. M., McMahon, A. C., Ballard, J. W. O., Ruohonen, K., Wu, L. E., Cogger, V. C., Warren, A., Huang, X., Pichaud, N., Melvin, R. G. et al. (2014). The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. *Cell Metab.* **19**, 418-430.

Solon-Biet, S. M., Walters, K. A., Simanainen, U. K., McMahon, A. C., Ruohonen, K., Ballard, J. W. O., Raubenheimer, D., Handelsman, D. J., Le Couteur, D. G. and Simpson, S. J. (2015). Macronutrient balance, reproductive function, and lifespan in aging mice. *Proc. Natl. Acad. Sci. USA* **112**, 3481-3486.

Troen, A. M., French, E. E., Roberts, J. F., Selhub, J., Ordovas, J. M., Parnell, L. D. and Lai, C.-Q. (2007). Lifespan modification by glucose and methionine in *Drosophila melanogaster* fed a chemically defined diet. *Age* **29**, 29-39.

Wheeler, D. (1996). The role of nourishment in oogenesis. *Annu. Rev. Entomol.* **41**, 407-431.

Young, Y., Buckiewicz, N. and Long, T. A. F. (2018). Nutritional geometry and fitness consequences in *Drosophila suzukii*, the Spotted-Wing *Drosophila*. *Ecol. Evol.* **8**, 2842-2851.