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RESEARCH ARTICLE

Linking developmental diet to adult foraging choice in *Drosophila melanogaster*

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ABSTRACT

Rather than maximizing intake of available macronutrients, insects increase intake of some nutrients and restrict intake of others. This selective consumption influences, and potentially optimizes, developmental time, reproduction and lifespan of the organism. Studies so far have focused on discriminating between protein and carbohydrate uptake and the consequences on fitness components at different life stages. However, it is largely unknown whether and how the developmental diets, which may entail habitat-specific nutrient restrictions, affect selective consumption in adults. We show that adult female *D. melanogaster* opt for the same protein to carbohydrate (P:C) ratio regardless of their developmental diet (P:C ratio of 1:1, 1:4 or 1:8). In contrast, males choose a diet that makes up for deficiencies; when protein is low during development, males increase protein consumption despite this being detrimental to starvation resistance. The sexual dimorphism in foraging choice could be due to the different energetic requirements of males and females. To investigate the effect of developmental diet on lifespan once an adult nutritional environment has been established, we also conducted a no-choice experiment. Here, adult lifespan increased as P:C ratio decreased, irrespective of developmental diet, thus demonstrating a 'cancelling out' effect of the nutritional environment experienced during early life stages. Our study provides novel insights into how developmental diet is linked to adult diet by presenting evidence for sexual dimorphism in foraging choice as well as life-stage dependency of diet on lifespan.

KEY WORDS: Sexual dimorphism, Lifespan, Nutritional choice, Developmental diet, Protein to carbohydrate ratio, Fitness, Starvation resistance

INTRODUCTION

The ability of insects to control the uptake of macronutrients is a well-documented phenomenon (reviewed by Simpson and Raubenheimer, 2012). Rather than maximizing intake of multiple nutrients when available, insects discriminate between intake of different nutrients (Simpson and Raubenheimer, 2012). This selective uptake has consequences for the organism's ability to maximize performance in fitness components such as developmental time, lifespan and lifetime egg production (Simpson and Raubenheimer, 2012).

Most of the knowledge we have on the consequences of a deficiency or surplus of a particular macronutrient in insects is derived from experiments investigating life-history and physiological consequences of exposure to fixed diets (where the organism has no choice) of different protein to carbohydrate (P:C) ratios. By providing the organism with a fixed macronutrient diet, no-choice experiments can identify the macronutrients that optimize a given trait. Such studies show that surpluses and/or deficiencies of protein and carbohydrate can optimize several traits, but also that they can be detrimental to others (Simpson et al., 2004; Lee et al., 2008, 2013; Christian et al., 2010; Fanson and Taylor, 2012; Bruce et al., 2013; Clark et al., 2013, 2015; Sentinella et al., 2013; Rodrigues et al., 2015; Runagall-McNaull et al., 2015). Sex, life stage and mating status all influence nutrient choice and thus the impact of nutrient intake might be highly context dependent (Simpson et al., 2004; Lee et al., 2002, 2008, 2013; Fanson et al., 2009; Andersen et al., 2010; Rodrigues et al., 2015). Experiments that have given the organism a choice have therefore allowed for a greater appreciation of how organisms balance macronutrients to achieve a desired diet (Lee et al., 2002, 2008, 2013; Simpson et al., 2004; Fanson and Taylor, 2012; Clark et al., 2015; Rodrigues et al., 2015).

One example of how macronutrient consumption is optimized is the influence of P:C ratio on lifetime fitness in the fruit fly, *Drosophila melanogaster*, and the Queensland fruit fly, *Bactrocera tryoni* (Lee et al., 2008; Fanson and Taylor, 2012). Females maximize their egg production at high P:C ratios but maximize their lifespan at low P:C ratios (Lee et al., 2008; Fanson and Taylor, 2012). When given a choice, both species opt for an intermediate ratio maximizing lifetime egg production. When *D. melanogaster* were given no choice, high amounts of protein intake shortened lifespan in both males and females (Lee et al., 2013). A surplus in protein ingestion can also have detrimental effects on lifespan in crickets (Maklakov et al., 2008), ants (Dussutour et al., 2012) and honeybees (Christian et al., 2010).

In many organisms, adult life-history traits are also influenced by developmental and early-life dietary experiences (Sentinella et al., 2013; Rodrigues et al., 2015; Runagall-McNaull et al., 2015). Protein restriction during adulthood typically prolongs lifespan, but when restricted access to protein occurs during the larval stage of the neriid fly *Telostylinus angusticollis*, it results in a reduction of lifespan (Runagall-McNaull et al., 2015). This suggests that if an organism is reared in an environment with access to constant nutrient resources across its entire lifespan, it will continuously adjust the relative uptake of macronutrients to maximize adult lifespan.

Because of this strong influence of developmental nutritional environment on fitness components, it has been suggested that organisms can respond to nutritional deficiencies or surpluses at earlier life stages by adjusting their nutrition preferences in the adult stage (Mevi-Schütz and Erhardt, 2003; Lee et al., 2012). Lee et al. (2012) showed that later-instar caterpillars exposed to diets with low protein during early life preferred more protein compared with those

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that had been exposed to higher protein diets. Studies on female map butterflies (*Araschina levana*) have also demonstrated compensatory feeding, with adults showing a preference for amino acids when developed on low-quality food (Mevi-Schütz and Erhardt, 2003). A later study found that these amino acids had a positive effect on fecundity and therefore this compensatory mechanism enables the butterflies to override the impact of poor larval food (Mevi-Schütz and Erhardt, 2005). Adjusting nutritional expenditure and desired diet later in life can allow organisms to survive through a range of nutritional fluctuations, but the lack of a nutrient at one life stage can be beneficial or detrimental at the next. It is therefore important for an organism to make the right foraging choices to enhance survival and maximize fitness.

The aim of the first part of this study was to assess how adult male and female *D. melanogaster* adjust their diet choices in response to macronutrient surpluses and deficiencies during larval stages. With previous experiments showing that feeding on different P:C ratios during development can cause trade-offs in fitness components (Rodrigues et al., 2015), we also wanted to investigate the effect of our developmental P:C ratios (1:1, 1:4 and 1:8) before an adult nutritional environment was established. To do this, egg-to-adult viability and starvation resistance were tested straight after emergence, before adult feeding. After developing *D. melanogaster* on P:C ratios of 1:1, 1:4 or 1:8, we measured the amount of protein and carbohydrate consumed by adults that could choose between two nutrients. Previous studies have shown that depriving larvae of either protein or carbohydrate causes the larvae to selectively feed on a diet to make up for the deficient macronutrient (Schwarz et al., 2014), but there is little understanding of how adult foraging choices are affected by deficiencies experienced during development. Because of the importance of balancing macronutrient intake across all life stages, we expected some level of compensatory feeding as a lack of one macronutrient throughout all life stages could have detrimental effects.

The second part of this study was performed to achieve a better understanding of how developmental diet affects adult lifespan once an adult nutritional environment has been established. In this experiment, flies were developed on the three P:C ratios and were transferred as adults to one of three diet ratios to analyse lifespan in a full factorial design. Previous experiments have shown that larvae reared in a protein-deficient environment have a short adult lifespan, while experiencing protein deficiencies in the adult life stage leads to a longer lifespan (Fanson and Taylor, 2012; Runagall-McNaull et al., 2015). Based on the reports of differences in the response to nutritional environment at different life stages, we hypothesized that adult diet, rather than developmental diet, would have the biggest effect on adult lifespan because of the apparent different nutritional needs of larvae and adults.

MATERIALS AND METHODS

Origin of flies

The *D. melanogaster* Meigen 1830 population used for this study originated from Odder, Denmark, and was established in the laboratory in 2010 (Schou et al., 2014). This population was maintained at 19°C on a 12 h:12 h light:dark photoperiod. Before the experiment, flies were kept for two generations at 25°C. In order to obtain eggs for transfer to the developmental diets, 1 day old flies (in groups of 10 adult males and 10 adult females, to control for density) were distributed into vials containing a spoon filled with agar and some yeast. Eggs were collected from the agar and placed in vials containing the developmental diets, avoiding transfer of any yeast to the new vial.

Experimental diets

For the experimental diets, we used instant dry yeast (instant yeast, LeSaffre, Marcq-en-Baroeul, France) and sucrose. The macronutrient values were calculated based on the yeast being made up of 98.5% *Saccharomyces cerevisiae* and 1.5% sorbitan monostearate, with nutrient values of 50% protein, 6% digestible carbohydrate, 27% indigestible fibre, 6% fats and 11% sodium, vitamin C, calcium and iron. As sorbitan monostearate does not affect the P:C ratio, protein and carbohydrate values were calculated based on the 98.5% *S. cerevisiae* content.

In the first experiment, adult flies were able to choose between two 5 µl microcapillary tubes, one containing a liquid diet of 180 g l^{-1} sucrose solution and the other containing a 180 g l^{-1} yeast solution (Ja et al., 2007). Developmental diet in both experiments and the no-choice adult diet in the second experiment were 2% agar based. The three P:C ratios used for the developmental diet and the no-choice adult diet were 1:1, 1:4 and 1:8, using yeast to sucrose ratios of 1:0.5, 1:2 and 1:4 at a concentration of 180 g l^{-1}. In addition to agar, yeast and sucrose, 12 ml l^{-1} of nipagen and 1.2 ml l^{-1} of acetic acid were used as anti-mould agents.

Developmental rearing and emergence of adults

Eggs were transferred to three distinct developmental diets containing P:C ratios of 1:1, 1:4 and 1:8. For each developmental diet, 40 vials containing 7 ml of medium and 20 eggs were produced. Because of differences in development times for flies reared on different P:C ratios (Rodrigues et al., 2015), eggs were placed on each developmental diet at 12 h intervals to ensure all adults emerged around the same time point. At 20:00 h, eggs were placed on the 1:8 diet; on the following day at 08:00 h, eggs were placed on the 1:4 diet; and at 20:00 h, eggs were placed on the 1:1 diet. Flies that had emerged overnight were discarded. Throughout the day, virgin flies were then collected every 4 h. Flies emerging at the peak emergence time were used for the feeding and starvation assays.

Choice assay and measurement of food intake

Within 6 h of emergence, flies were placed individually into a vial containing 3.5 ml of agar and the two microcapillary tubes (containing the sucrose or yeast solution). Intake was measured using the height difference between the tube and remaining liquid. The vials were placed in a sealed tank containing water to maintain a high humidity and limit evaporation of the liquid diet from the capillary tubes; however, to account for evaporation, vials set up in exactly the same way but containing no fly were also placed in the tank. Flies were kept under these conditions for 4 days, with fresh capillary tubes being provided at the end of the second day. Flies ingest a volume of 1.3–2.3 µl per day, so by providing the fly with fresh tubes at the end of the second day, we ensured that flies did not run out of food (Ja et al., 2007).

Egg-to-adult viability

Egg-to-adult viability was measured by randomly transferring 20 eggs from the density-controlled parental vials into vials containing 7 ml developmental medium. The total number of adults that emerged was counted across the replicate vials for each of the three developmental diets. The viability was measured as the percentage of adults that emerged from around 440 eggs for each diet.

Starvation resistance

The first of two starvation resistance assays was performed on newly emerged flies to assess the effect of developmental diet on starvation resistance. Within 4 h of emergence, approximately 22

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flies of the same age of each sex from each developmental diet were placed individually in a vial containing 3.5 ml of a water and agar solution. Every 8 h, the number of dead flies was counted until all flies had died. The second starvation resistance assay was performed using exactly the same method, but on flies which had experienced 4 days of feeding, to assess the effect of the flies' chosen diet on starvation resistance.

Lifespan

To assess the effect of developmental and adult diet on lifespan, a full factorial design using the three diets (P:C ratios of 1:1, 1:4 and 1:8) was used. After developing on one of the three developmental diets with P:C ratios of 1:1, 1:4 or 1:8, adult flies were placed on a diet with P:C ratios of 1:1, 1:4 or 1:8 within 8 h of emergence. Food was prepared using the same method as for the developmental diet and 3.5 ml of food was placed in each vial. For each of the nine treatments, males and females were separated into groups of approximately 10; this was replicated 10 times (for the exact number of flies per vial, see Table S1). The number of dead flies in a vial was counted every other day and the remaining living flies were placed in a fresh vial to reduce the risk of bacterial growth.

Statistical analysis

All statistical analyses were carried out in RStudio (R Studio Team 2016, https://www.rstudio.com/), with developmental diet (P:C ratios of 1:1, 1:4 and 1:8) being treated as a continuous variable (1, 0.25 and 0.125, respectively). Assumptions for parametric analysis were fulfilled in all models.

To investigate the effect of developmental diet on egg-to-adult viability, we used a logistic regression in a generalized linear model. The model contained developmental medium as the only predictor variable. We detected no over-dispersion in the model. We compared the full model with a reduced model from which developmental diet was omitted, using a likelihood ratio test to obtain a *P*-value for the effect of developmental diet.

The effect of developmental diet on protein and carbohydrate intake during adulthood and on starvation resistance before and after 4 days of feeding was assessed by constructing separate linear models. The models contained the given response variable (protein or carbohydrate intake), as well as the predictor variables developmental medium and sex (males and females) and the interaction between the two. The full models were compared with reduced models without the interaction using an *F*-test. In the case of a significant interaction, we split the dataset into male and female subsets and evaluated the effect of developmental medium for each sex separately using *F*-tests.

The effect of protein intake on starvation resistance was investigated with a linear model. The model contained the predictor variables sex and protein intake as well as the interaction between the two. A similar approach was used to investigate the effect of carbohydrate on starvation resistance. To obtain *P*-values of the model components, we performed sequential model reduction and compared models using *F*-tests.

To investigate the effect of developmental medium and adult medium on lifespan, we used a linear model with the median lifespan of each vial as the response variable. The model contained the predictor variables developmental medium, adult medium, sex and all possible interactions. To obtain *P*-values of the model components, we performed sequential model reduction and compared models using *F*-tests.

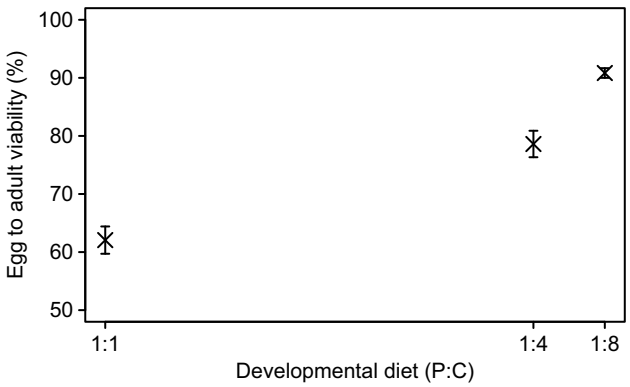

Fig. 1. Egg-to-adult viability. Mean (±s.e.m.) percentage of adult *Drosophila melanogaster* that emerged from 20 eggs developed on a diet with protein to carbohydrate (P:C) ratios of 1:1, 1:4 or 1:8 (number of vials containing 20 eggs: *n*=17, *n*=18, *n*=18, respectively).

RESULTS

Egg-to-adult viability and starvation resistance before adult feeding

Developmental diets at decreasing P:C ratios of 1:1, 1:4 and 1:8 caused an increase in egg-to-adult viability (χ^2=122.5, *P*<0.001) (Fig. 1). Flies emerging from the three developmental diets were sexed and transferred to the first of the starvation resistance assays. For starvation resistance, there was no interaction between developmental diet and sex ($F_{1,130}$=1.658, *P*=0.201), and therefore we combined the data from males and females, and found an increase in starvation resistance as developmental P:C ratio decreased ($F_{1,131}$=77.008, *P*<0.001) (Fig. 2).

Foraging choices

When flies were given the choice of yeast (a protein source) and sugar (a carbohydrate source) solutions, a significant interaction effect on their foraging choice was found between developmental diet and sex for protein intake and P:C ratio intake (Fig. 3, Table 1). As P:C ratio decreased during development, adult males increased their P:C intake by increasing their protein intake (Fig. 3A,C, Table 1). In adult females, in contrast, the three different developmental diets affected neither their protein intake nor their P:C intake (Fig. 3A,C, Table 1). Developmental diet did not significantly change carbohydrate intake ($F_{1,122}$=2.782, *P*=0.098)

Fig. 2. Starvation resistance before adult feeding. Mean (±s.e.m.) starvation resistance of newly emerged female and male *D. melanogaster* developed on a diet with P:C ratios of 1:1, 1:4 or 1:8 (females: *n*=21, *n*=22, *n*=21; males: *n*=23, *n*=23, *n*=23, respectively).

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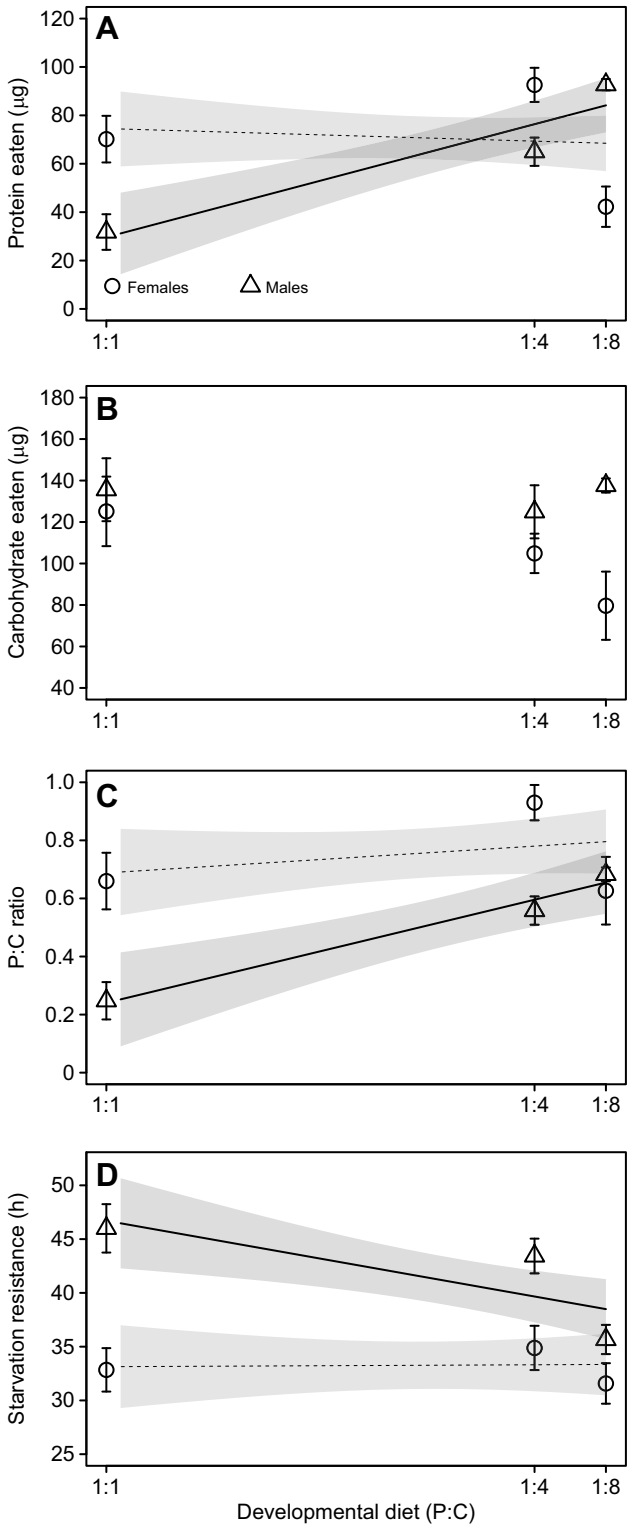

Fig. 3. Foraging choice and starvation resistance after adult feeding. Mean (±s.e.m.) adult nutrient intake and starvation resistance of *D. melanogaster* developed on a diet with P:C ratios of 1:1, 1:4 or 1:8 (females: n=19, n=25, n=19; males: n=16, n=21, n=24, respectively). (A) Protein intake. (B) Carbohydrate intake. (C) P:C ratio of adult nutrient intake. (D) Starvation resistance after 4 days of feeding. When there was a significant effect of sex and developmental diet, the fitted lines represent the predicted response based on the linear model (dotted line, female; solid line, male) with the shaded area representing the 95% confidence interval.

Table 1. Results for the nutrient intake and starvation after adult feeding assay from linear model ANOVA as a response to developmental diet and sex

	Developmental diet		
	Estimate±s.e.	$F_{d.f.}$	P-value
Developmental diet×sex			
Protein eaten	6.935±11.845	$16.360_{1,121}$	<0.001***
Carbohydrate eaten	–	$2.060_{1,121}$	0.154
P:C ratio	−0.123±0.114	$4.56_{1,121}$	0.035*
Starvation after adult feeding	−0.238±2.956	$5.119_{1,121}$	0.025*
Female			
Protein eaten	–	$0.228_{1,62}$	0.634
Carbohydrate eaten	–	–	–
P:C ratio	–	$0.717_{1,62}$	0.400
Starvation after adult feeding	–	$0.006_{1,62}$	0.940
Male			
Protein eaten	−62.273±8.577	$52.717_{1,60}$	<0.001***
Carbohydrate eaten	–	–	–
P:C ratio	−0.0473±0.07	$45.255_{1,60}$	<0.001***
Starvation after adult feeding	9.389±2.841	$10.925_{1,60}$	0.002**

Four different response variables were assessed for an interaction between developmental diet and sex [protein eaten, carbohydrate eaten, protein: carbohydrate (P:C) ratio and starvation after adult feeding]. In the case of a significant interaction, separate models for males and females were constructed to test for an effect of developmental diet. Data from the analysis of starvation before adult feeding are presented in the Results.
Asterisks indicate the level of significance.

(Fig. 3B, Table 1). Overall, the male intake of carbohydrate was higher than that of females ($F_{1,122}$=8.866, P<0.05). Females overall chose a higher P:C ratio intake compared with males across the three developmental diets ($F_{1,121}$=13.024, P<0.001).

Starvation resistance after adult feeding

The second starvation resistance assay was performed after 4 days of feeding and a significant interaction between developmental diet and sex was found (Table 1). Female starvation resistance after 4 days of feeding followed the same pattern as nutrient intake, with no change across developmental P:C ratio (Fig. 3D, Table 1). In males, however, mean starvation resistance decreased as the developmental P:C ratio decreased (Fig. 3D, Table 1). Overall, males survived starvation significantly longer than females ($F_{1,121}$=23.131, P<0.001).

Further, we investigated how the protein and carbohydrate intake correlated with starvation resistance in males and females. No significant interaction was found between sex and protein intake ($F_{1,121}$=0.088, P=0.768) and sex and carbohydrate intake ($F_{1,121}$=2.951, P=0.088). For both males and females, starvation resistance decreased when the intake of protein increased ($F_{1,121}$=0.316, P<0.001). Carbohydrate intake did not have a significant effect on starvation resistance in either sex ($F_{1,121}$=1.015, P=0.316).

Lifespan

In the no-choice foraging experiment, we did not observe significant interactions between developmental diet, adult diet and sex and the pairwise interactions (Table 2). No effect of developmental P:C ratio on lifespan was found (Table 2, Fig. 4). A difference in lifespan was seen between the adult diets; as adult P:C ratio decreased, lifespan increased (Table 2, Fig. 4). Overall, female lifespan was significantly longer than that of males (Table 2).

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Table 2. Results from the sequential model reduction for adult lifespan

Effect	Estimate±s.e.	$F_{d.f.}$	*P*-value
Intercept	43.646±1.150	–	–
Sex (males)	−6.931±0.977	$106.290_{1,174}$	<0.001***
Adult diet	−11.956±1.126	$184.13_{1,174}$	<0.001***
Developmental diet	–	$2.419_{0,173}$	0.122
Adult diet×sex	–	$0.615_{1,171}$	0.434
Developmental diet×sex	–	$0.347_{1,170}$	0.557
Developmental diet×adult diet	–	$2.762_{1,172}$	0.984
Developmental diet×adult diet×sex	–	$1.464_{1,169}$	0.228

Asterisks indicate the level of significance.

DISCUSSION

Effects of developmental P:C ratios

The influence of different proportions of protein and carbohydrate ingested during development has previously been tested in insects to investigate trade-offs between developmental time, egg-to-adult viability and morphological traits (Sentinella et al., 2013; Nash and Chapman, 2014; Rodrigues et al., 2015). The first part of our study investigated the effect of developmental P:C ratios on egg-to-adult viability and starvation resistance before adult feeding. We found no trade-off between the two traits. Both viability and starvation resistance increased as developmental P:C ratio decreased. Our results support those of Chippindale et al. (1993), who showed that rearing larvae on a high concentration of yeast decreased starvation resistance compared with that of larvae reared on low amounts of yeast. However, Rodrigues et al. (2015) found that egg-to-pupae survival was optimized at P:C ratios of around 1:1–1:5. The discrepancy between the results could be due to differences in the response to nutrient intake when different protein sources are used (Lee et al., 2008, 2013) as well as genetic differences between the populations studied.

Adult foraging choice and starvation resistance

In order to investigate how adult flies balance their protein and carbohydrate intake in response to developmental diets with different P:C ratios, we provided adults with a choice between a yeast solution and a sugar solution. We found that in females, adult P:C preference was not affected by developmental diet. Males, in contrast, responded to protein deficiencies in the developmental diet by increasing their adult P:C ratio intake. By correlating nutrient intake with starvation resistance, we also showed that the intake of protein, but not of carbohydrate, was negatively correlated with starvation. Our results are consistent with those of Simmons and Bradley (1997) and Lee and Jang (2014), who both found a significant decrease in starvation resistance with higher protein intake. Lee and Jang (2014) showed that flies were better able to resist starvation by storing more lipids when consuming diets richer in carbohydrate. Based on these observations, they concluded that increased P:C ratio limits lipid storage. Our study thus supports that balancing protein and carbohydrate intake is important for starvation resistance and that neither caloric value nor carbohydrate intake alone is important for sustaining periods without access to food.

Previous studies have also found benefits of protein-rich diets for males at both the developmental and the adult stage (McGraw et al., 2007; Fricke et al., 2008). By investigating time to copulation with a female, McGraw et al. (2007) showed that males developed on diets richer in protein sired more offspring than males developed on lower amounts of protein. Increasing protein content in the diet during adulthood also increased the number of offspring sired (Fricke et al., 2008). Therefore, despite reduced starvation resistance of flies developed on a protein-rich diet, reproductive success seems to be enhanced and increased protein intake might be a strategy to ensure mating success.

Our data showed that females did not change their foraging choices across the three developmental diets. Despite virgin females and males having previously been shown to select similar diets (Lee et al., 2013), the physiological differences between the sexes would probably result in different adaptive strategies and nutrient allocations across the life stages. Whereas male *D. melanogaster* simply make up for deficiencies, females may need to stick to a ‘standard’ diet during adulthood in order to balance the trade-off between egg production and lifespan (Lee et al., 2008). When given a choice, female flies chose a diet that balanced the lifespan-lengthening effects of carbohydrate and the positive effects of protein on egg production to maximize lifetime egg production (Lee et al., 2008). Making up for deficiencies from the

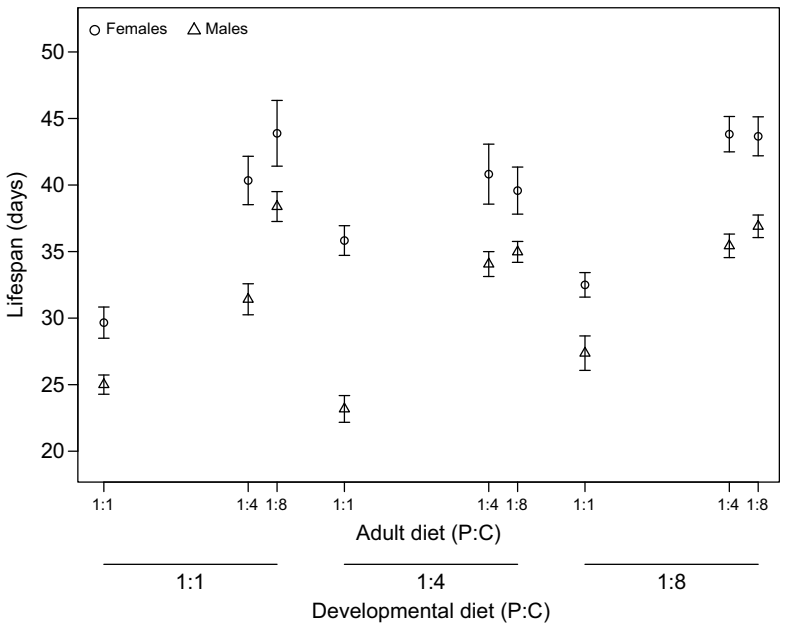

Fig. 4. Lifespan. Mean (±s.e.m.) lifespan of male and female *D. melanogaster* developed on a diet with P:C ratios of 1:1, 1:4 or 1:8 and then fed a diet with P:C ratios of 1:1, 1:4 or 1:8 during adulthood.

developmental diet, as males did, could jeopardize this important balancing of macronutrients and have detrimental effects on the balance between lifespan and egg production.

The ability to adjust diet choices throughout life allows a higher chance of surviving through periods with fluctuating nutritional availability occurring on temporal and spatial scales in nature. The sex specificity observed is probably due to the differences between males and females in terms of the energy needed for reproduction and may have a background in sexual selection. For males, there are energetic needs for courtship movements as well as macronutrient needs for the production of accessory gland proteins (Droney, 1998; Fricke et al., 2008; Maklakov et al., 2008). This would, therefore, lead to males opting for the most effective diet to obtain maximum energy to perform courtship movements and for post-copulatory mating success. Failure to do this would result in less success in female choice and male competition, i.e. sexual selection.

Consistent with previous findings (Aguila et al., 2007), our two starvation assays showed that newly emerged flies survived overall, on average, 20 h longer without food than older flies (in this case, 4 days old after feeding). In nature, organisms can face periods of food shortages where the ability to survive starvation stress is an important fitness component. Once emerged from the pupae, flies will be faced with a non-feeding period as they anatomically develop further and find a food source. Larval fat cells that remain in the fly during early adulthood act as 'transporters' of nutrients ingested during larval stages and therefore provide an important source of energy (Aguila et al., 2007). Aguila et al. (2007) also showed the number of larval fat cells that are depleted during adult development positively correlates with starvation survival, i.e. as adults get older, their ability to survive starvation decreases. This is in accordance with the decreased starvation resistance of 4 day old adults observed in this study.

Effect of P:C ratio on lifespan

By conducting a no-choice experiment, we were able to assess the importance of developmental diet, adult diet and the combination of both on lifespan. Our data showed that as adult diet P:C ratio decreased, lifespan increased, supporting previous studies showing high protein content in diets had a negative effect on lifespan (Lee et al., 2008, 2013; Fanson et al., 2009; Bruce et al., 2013). Although not measured in this study, varying the developmental P:C ratio affects developmental time and growth rate (Rodrigues et al., 2015). Varying larval density and food quality has been seen to have the same effect (Zwaan et al., 1991). In both the present study and the study by Zwaan et al. (1991), larval environment influencing pre-adult growing traits did not influence adult lifespan. Both these results suggest that fitness consequences due to developmental environment can be 'cancelled out' during adulthood.

Conclusion

This study is the first (to our knowledge) to provide an insight into how developmental diet in *D. melanogaster* is linked to adult foraging choices and therefore adult diet. We found that males and females differ in foraging choices, highlighting the importance of sex-specific nutritional needs, and our results provide insights into the importance of diet choice on sexual selection mechanisms. By conducting a no-choice experiment, we also showed that the developmental nutritional environment had no lasting effects on lifespan once an adult nutritional environment had been established.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: L.R.D., M.F.S., V.L.; Methodology: L.R.D., M.F.S., V.L.; Validation: L.R.D.; Formal analysis: L.R.D., M.F.S.; Investigation: L.R.D.; Resources: T.N.K., V.L.; Writing - original draft: L.R.D.; Writing - review & editing: L.R.D., M.F.S., T.N.K., V.L.; Supervision: T.N.K., V.L., M.F.S.; Project administration: V.L.; Funding acquisition: T.N.K., V.L.

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Data availability

Phenotypic data are available from figshare: https://figshare.com/projects/Linking_developmental_diet_to_adult_foraging_choice_in_Drosophila_melanogaster/31898

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.175554.supplemental

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Supplementary material

Table S1. Number of flies per vial for lifespan experiment. Flies were initially grouped in 10, however some were lost when being transferred to new vials.

FEMALE	**Dev. diet**			**MALE**	**Dev. diet**		
Adult diet	1:1	1:4	1:8	**Adult diet**	1:1	1:4	1:8
Vial 1				**Vial 1**			
1:1	9	6	8	1:1	7	6	10
1:4	10	0	10	1:4	7	9	10
1:8	7	7	6	1:8	8	8	9
Vial 2				**Vial 2**			
1:1	8	6	9	1:1	10	6	7
1:4	9	6	10	1:4	8	9	9
1:8	8	7	7	1:8	7	7	8
Vial 3				**Vial 3**			
1:1	8	7	5	1:1	9	10	7
1:4	7	7	3	1:4	9	7	9
1:8	8	6	8	1:8	6	10	10
Vial 4				**Vial 4**			
1:1	8	8	7	1:1	0	10	9
1:4	9	7	6	1:4	7	10	8
1:8	0	6	7	1:8	7	8	9
Vial 5				**Vial 5**			
1:1	10	7	7	1:1	9	8	9
1:4	9	9	7	1:4	9	8	7
1:8	7	8	8	1:8	6	10	6
Vial 6				**Vial 6**			
1:1	8	9	9	1:1	8	7	6
1:4	4	8	7	1:4	6	6	9
1:8	7	9	8	1:8	7	6	9
Vial 7				**Vial 7**			
1:1	9	9	8	1:1	10	10	6
1:4	8	6	9	1:4	6	9	10
1:8	8	9	9	1:8	6	8	7
Vial 8				**Vial 8**			
1:1	8	8	10	1:1	10	8	5
1:4	10	10	8	1:4	6	6	8
1:8	8	8	4	1:8	9	9	8
Vial 9				**Vial 9**			
1:1	7	5	9	1:1	5	6	6
1:4	9	4	10	1:4	7	6	7
1:8	7	7	5	1:8	0	7	9
Vial 10				**Vial 10**			
1:1	9	6	8	1:1	8	8	10
1:4	10	0	10	1:4	10	8	9
1:8	7	7	6	1:8	7	7	8