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Sexual dimorphism in nutrient intake and life span is mediated by mating in *Drosophila melanogaster*

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Mating elicits dramatic changes in behaviour and physiology related to reproduction in female *Drosophila melanogaster*, but little is known about how mating affects nutrient intake and preferences in this model organism. Postmating switches in reproductive activity and feeding are likely to alter life span because both reproduction and nutrition are closely linked to survival. To explore these possibilities, we compared the intake patterns of protein (P) and carbohydrate (C) preferred by male, mated female and virgin female adults of *D. melanogaster* and their life span responses under varying dietary P:C balances (0:1, 1:1 and 4:1). Despite sex differences, the patterns of both macronutrient intake and life span were remarkably similar between males and virgin females. Compared to unmated virgins, mated females consumed ca. 2.7 times more nutrients and preferred a diet that was significantly more protein-rich (mated versus virgin females: P:C 1:1.5 versus 1:4). Surprisingly, females that had experienced mating in early adulthood lived longer than those that remained virgin, and this life span-extending effect of mating in females was most profound on a diet that was equally balanced (P:C 1:1). Our results indicate that mating is an important modulator that regulates sex-specific nutrient preference, which in turn alters longevity in *D. melanogaster*.

Longevity and reproduction are of fundamental importance to all living organisms and both depend vitally upon the balanced intake of multiple nutrients (Simpson & Raubenheimer 2012). It is well established that males and females require different amounts and balance of nutrients to maximize their reproductive output (Maklakov et al. 2008; Lee 2010; Morehouse et al. 2010). Sex differences in life span are also ubiquitous across taxa (Clutton-Brock & Isvaran 2007) and each sex is known to respond differently to genetic and environmental interventions that affect life span (Spencer et al. 2003; Burger & Promislow 2004; Magwere et al. 2004; Bross et al. 2005), perhaps because of sex differences in nutrient acquisition and allocation and in molecular mechanisms that control ageing (e.g. insulin/insulin-like growth factor-1 signalling pathway). As indicated by an earlier study by Magwere et al. (2004), the extent to which life span was prolonged with moderate dietary restriction was much greater in females than in males in *Drosophila melanogaster*. Hence, to understand better the complex relationship between life span and reproduction, it is essential to delineate the nutritional basis that underlies sex differences in these two key life history components.

Mating is an integral part of reproduction in all sexually reproducing animals, allowing sperm transfer and egg fertilization. In addition, mating can be a critical turning point in an animal's life in many species by triggering irreversible switches in development, physiology and behaviour of sexually mature females. For instance, it has been observed from adult female *D. melanogaster* that mating stimulates egg production, reduces survival, represses sexual receptivity, increases locomotor activity and switches feeding behaviour. Several lines of evidence have suggested that these postmating responses are regulated by the male seminal fluid compounds that are transferred to the female during mating (Chen et al. 1988; Herndon & Wolfner 1995; Kubil 2003; Carvalho et al. 2006; Barnes et al. 2008; Isaac et al. 2010). Evidence from a wide range of taxa including *D. melanogaster* has shown that animals are capable of adjusting their diet preference to satisfy the optimal requirement of multiple nutrients (Lee et al. 2008; Simpson & Raubenheimer 2012), which changes dynamically with development and physiological status (Barton Browne 1995). Therefore, an emerging issue to be resolved is: what are the quantitative and qualitative effects of mating on feeding behaviour in female *D. melanogaster*?

Our first aim in this study was to investigate how adult *D. melanogaster* regulate their nutrient intake according to sex and mating status. Since life span is an important life history parameter that is inevitably linked to nutrient intake in *D. melanogaster* (Lee

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et al. 2008; Grandison et al. 2009a), the second aim of our research was to determine whether there is any coupling between changes in the pattern of life span and food intake mediated by sex and mating. Despite its status as a model organism, research on the nutritional biology of *D. melanogaster* is still in its infancy, largely because of a lack of appropriate experimental and analytical methods for assessing nutrient intake in this species (but see Lee et al. 2008). Most published studies on nutrition in *D. melanogaster* have used semisynthetic diets with yeast as the main dietary source for protein (Bass et al. 2007) and have often used surrogate measures of food intake such as faecal deposits, proboscis extensions, dye uptake, calorimetry and radiotracers (Edgecomb et al. 1994; Carvalho et al. 2005; Mair et al. 2005; Min et al. 2007; Wong et al. 2009). Because yeast contains diverse nutrients other than protein (e.g. carbohydrates, sterols, fatty acids, minerals, vitamins, etc.), there has been a growing demand among investigators for developing a standardized synthetic diet for *D. melanogaster* to disentangle the complex interactions between multiple nutrients affecting this species (Grandison et al. 2009b; Simpson & Raubenheimer 2009; Piper et al. 2011). In our study we took a nutritionally explicit approach that differs from previous ones in the following three ways. First, we introduced chemically defined synthetic diets designed for *D. melanogaster*, which enabled us to manipulate experimentally the precise quantity and mixture of dietary components. Second, unlike most studies that estimated food intake from surrogate measures, we quantified the amounts of nutrients actually ingested by individual flies by using capillary feeder assay (known as CAFE assay), a recently developed technique that has proved to be a powerful tool for measuring food intake in *D. melanogaster* (Ja et al. 2007; Lee et al. 2008). Third, we used the geometric framework (GF) for nutrition as our analytical platform. The GF is an integrative, state-space modelling program developed for investigating the intricate relationships between nutrition and various aspects of the biology of animals (Simpson & Raubenheimer 2012). This approach offered us a unique opportunity to visualize how the two sexes of *D. melanogaster* differed in their choice of nutrient ingestion with unprecedented high resolution (Lee et al. 2008; Simpson & Raubenheimer 2012). We formulated the following three hypotheses. (1) Females prefer protein over carbohydrate to a greater extent than males. We predicted this because protein is required for egg production. (2) Female-specific preference for protein is expressed more profoundly in females that experienced mating than in virgins. We predicted this because mating stimulates egg production in female *D. melanogaster*. (3) Any differences in nutrient intake caused by sex difference or mating status are likely to result in correlated alterations in life span.

METHODS

Experimental Flies

Wild-type, outbred, Canton-S *D. melanogaster* flies were cultured on a standard larval rearing medium (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 per 1 litre distilled water) at 25 °C under a light:dark cycle of 12:12 h at Seoul National University for more than 25 generations prior to this experiment. To obtain flies for the experiment, more than 1000 adult flies aged less than a week were released into a population cage (210 × 410 mm and 210 mm high) and given oviposition substrates (4% agar, 10% molasses covered with live yeast paste in a 9 cm diameter petri dish) and a water source (distilled water in a 150 ml plastic bottle plugged with water-soaked cotton). After 3 days of acclimation in this egg-laying environment, flies were given fresh oviposition substrates and allowed to lay eggs there for 4 h. Eggs laid on the substrate surface were washed with phosphate-buffered saline (PBS) and harvested using a mesh filtering method. Collected eggs were then dispensed into 1.5 ml Eppendorf centrifuge tubes filled with PBS solution where they precipitated to the bottom. Using a micropipette, we added 20 μl of egg precipitate to each fly culturing bottle containing 20 ml of standard medium, and this allowed a constant larval rearing density of 250–300 flies per bottle (Clancy & Kennington 2001).

Experimental Diets

Chemically defined, synthetic diets for adult *D. melanogaster* were prepared by modifying the method described by Roberts (2003). In the current study, protein (P) and digestible carbohydrate (C) were the two macronutrients that were experimentally manipulated into three dietary P:C balances of 0:1, 1:1 and 4:1. This range of ratios was chosen to represent the nutritional composition of the diets consumed by *D. melanogaster* in both natural and laboratory conditions (e.g. from sugar to yeast). The concentration of protein plus carbohydrate (P + C) of all three diets was fixed to 12 g per 100 ml medium (i.e. 120 g/litre). Sodium caseinate (Sigma C8654) and sucrose (Sigma S9378) were used as the sources for protein and carbohydrate in our synthetic diets, respectively. Apart from these two nutrients, all three diets contained constant amounts of dietary lipids (0.03 g cholesterol and 0.4 g lecithin), salts (7.1 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) and preservatives (0.1 g nipagin and 0.3 ml propionic acid) per 100 ml medium. Each P:C balance diet was prepared in both solid and liquid form. To produce solid medium, all preweighed dry ingredients except vitamins and preservatives were dissolved homogeneously in sterile, distilled water and 2.0–2.5 g of agar powder was added to this diet mix solution before autoclaving at 15 IB for 10 min. After autoclaving, vitamins and preservatives were added to the medium when it had cooled to <50 °C and the final volume of the medium was set to 100 ml by adding distilled water. After vigorous stirring, solid diets were dispensed into 20 ml fly vials in 4 ml aliquots, stabilized at room temperature for 4 h, and stored in a cold laboratory chamber at 4 °C until use. Liquid diet solutions were prepared using the same protocol as the solid diets, but without the addition of agar powder.

Experiment 1: Sex-specific Effects on Nutrient Intake

Freshly emerged flies of both sexes were collected within 4 h of eclosion, ensuring that no flies had mated before the point of collection. These newly eclosed flies were sexed under light CO_2 anaesthesia and assigned to one of two pre-experimental conditions: (1) a mating treatment in which newly eclosed male and female flies were housed together and allowed to mate in culturing bottles containing 20 ml of standard rearing medium (40–50 flies for each sex per bottle) and (2) a nonmating treatment in which only the cohorts of unmated, virgin females were released into the same rearing bottles (80–100 virgin females per bottle). After 48 h, flies from these treatments were individually transferred to 6 ml plastic vials (day 0), and divided into three experimental groups: (1) males, (2) mated females and (3) virgin females. We implemented the CAFE assay to quantify actual food intake for individual flies (Lee et al. 2008). In brief, each fly in the vial was simultaneously provided with two 5 μl microcapillary tubes (Drummond Microcaps), one filled with liquid diet solution that contained four times more protein than carbohydrate (P:C = 4:1) and the other

with a solution that comprised only carbohydrate (0:1; $N = 22–25$ replicates per group). We dispensed 1 ml of 1% agar solution (distilled water) to each vial to provide an ad libitum water source. Microcapillary tubes were replaced every 3 days and more frequently if they were emptied earlier. The intake of liquid diet solution by individual flies over 3 days was measured against a scale bar by the height difference in the column of liquid within each tube. We ensured that humidity was constantly high in the environment to minimize evaporative loss of liquid diet solution. None the less, control microcapillaries were used to correct for any evaporation that may influence the estimation of nutrient consumption for each liquid diet ($N = 12$). To measure egg production rate, we set up a separate subgroup of female flies ($N = 14$ for mated and virgin females) confined to the same individual vials as described above. Eggs laid by each fly were counted at 3-day intervals until day 9. Empty egg shells were also counted and added to the total egg count, providing an accurate measure of eggs laid over each 3-day interval.

Experiment 2: Sex-specific Effects on Life Span

Several thousand freshly eclosed flies were collected and allocated to either mating or nonmating preconditioning treatments following the same protocol as previously described. After 48 h of the mating treatment, an initial cohort size of ca. 100–120 individuals was placed in each 1-litre demography cage for (1) males, (2) mated females and (3) virgin females. Each demography cage had two side-arm inlets that can simultaneously accommodate two 20 ml fly vials, one containing a 4 ml aliquot of solid diet medium from one of the three dietary P:C balance treatments (0:1, 1:1 and 4:1) and the other containing the same volume of distilled water (with 1% agar). The latter was provided to eliminate any confounding effects on life span from dehydration (Ja et al. 2009). The synthetic diets and water source were replenished every 2 days and dead flies were counted and removed daily. For the life span assay, there were four to five replicate demography cages per experimental group and dietary treatment, with a total of ca. 1800 flies being used for the whole experiment. Measurement of life span was continued until the death of the last remaining individual. The experimental chamber was maintained at 25 °C with a light:dark cycle of 12:12 h and a humidity >70%. The positions of the demographic cages in the chamber were rotated twice a day to avoid the effects of microclimate. Egg production rate was measured separately from life span by allocating a group of one female and two male flies to 20 ml fly vials that contained one of the three solid diets (0:1, 1:1 and 4:1). Each fly group was then allowed to acclimate to these new egg-laying conditions for 5 days before the first egg count. After this acclimation period, flies were transferred to a vial containing fresh medium and eggs laid by each female were counted daily for 5 consecutive days. Fresh medium was provided on a daily basis during this egg count period. We used the total number of eggs produced by female flies over the 5 days as the measure for egg production rate.

Statistical Analyses

We used univariate and multivariate analysis of variance (ANOVA and MANOVA) to test for overall differences in nutrient intake between males, mated females and virgin females. For MANOVA, we used Pillai's trace statistic. Before conducting these parametric analyses, we checked normality and homoscedasticity using Kolmogorov–Smirnov and Bartlett's test, respectively. Cox regression analysis was conducted to investigate the effects of sex treatment (male, mated female and virgin female) and dietary P:C balance (0:1, 1:1 and 4:1) on life span. Egg count data were analysed using appropriate nonparametric tests. All statistical analyses were performed using SAS v 9.1 statistical software (SAS Institute, Cary, NC, U.S.A.).

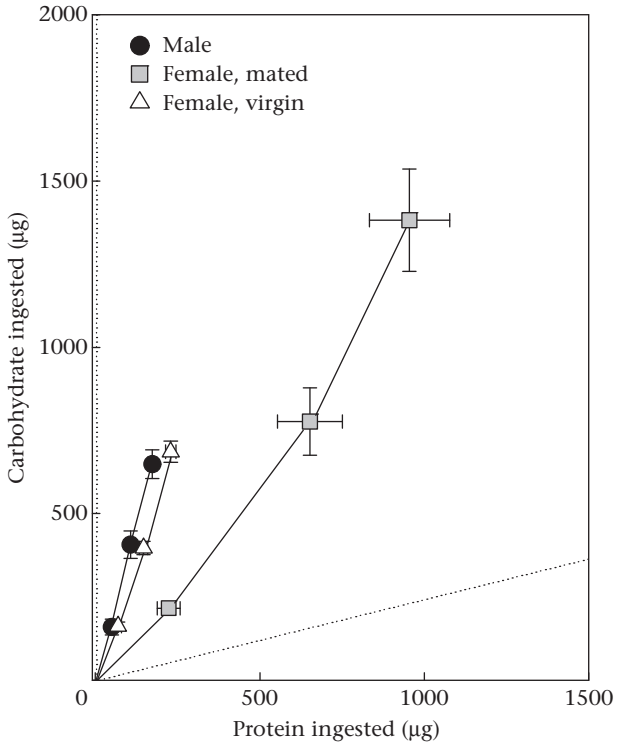

Figure 1. Cumulative patterns of protein and carbohydrate intake (mean ± SEM) self-composed by male, mated female and virgin female *D. melanogaster* given a choice between two diets with nutritionally complementary P:C balance (0:1 and 4:1) over days 0–3, 0–6 and 0–9. Solid lines represent the nutrient intake trajectories for each fly group. Dotted lines are rails that represent the P:C balance of the two choice diets (0:1 and 4:1).

RESULTS

Experiment 1: Sex-specific Effects on Nutrient Intake

When the pattern of nutrient intake was examined over days 0–9 (Fig. 1), we found that mated females not only consumed ca. 2.7-fold more macronutrients (ANOVA: $F_{2,64} = 29.69$, $P < 0.001$), but also self-composed a diet containing a significantly higher ratio of protein to carbohydrate (P:C) than males and virgin females (1:1.5 versus 1:4; ANOVA on P:C ratios arctangent-transformed into radians: $F_{2,64} = 48.57$, $P < 0.001$). A post hoc Tukey multiple comparison test revealed that virgin females were quantitatively and qualitatively indistinguishable from males, with the two types of flies ingesting similar amounts and mixture of nutrients to each other (Fig. 1). Results from MANOVA contrasts on the bivariate pattern of protein–carbohydrate intake confirmed this relationship, demonstrating that the nutrient intake of virgin females closely resembled that of males but differed significantly from that of mated females (Table 1). During the period of food choice, the median numbers of eggs laid by mated and virgin females were 6 and 0, respectively (Mann–Whitney test: $Z = -4.07$, $N_1 = N_2 = 14$, $P < 0.001$).

Experiment 2: Sex-specific Effects on Life Span

Life spans were significantly affected by sex treatment group (Cox regression: $\chi^2_2 = 20.94$, $P < 0.001$) and by dietary P:C balance

Table 1
Results of MANOVA on cumulative protein and carbohydrate intake over days 0–3, 0–6 and 0–9

Source	*df* (hypothesis, error)	*F* ratios		
		Days 0–3	Days 0–6	Days 0–9
MANOVA				
Experimental group	4,128	7.82***	10.45***	12.20***
Multivariate contrasts				
Virgin female versus male	2,63	0.24	0.44	0.20
Virgin female versus mated female	2,63	12.41***	18.7***	25.01***

Pillai's trace *F* ratios are reported for overall MANOVA and for multivariate contrasts between specific experimental groups. The three experimental groups comprise adult male, mated female and virgin female *D. melanogaster*.
***$P < 0.001$.

($\chi^2_2 = 162.78$, $P < 0.001$), with a significant interaction between these two main effects ($\chi^2_4 = 112.20$, $P < 0.001$). Regardless of sex and mating status, flies fed the protein-rich diet (P:C balance, 4:1) had the shortest life span, with the median life spans of all three groups on this diet being 16–17 days and statistically indistinguishable from one another ($\chi^2_2 = 3.01$, $P = 0.223$; Fig. 2). When dietary P:C balance was either equally balanced (1:1) or carbohydrate-rich (0:1), mated females lived longer than both males and virgin females (Fig. 2), but the extent to which life span was prolonged with mating in females was much greater when dietary P:C balance was 1:1 than when it was 0:1 (64% versus 21% increase in median life span; Fig. 2). Life spans of males and virgin females were similar over the range of dietary P:C balances ($\chi^2_1 = 0.63$, $P = 0.428$). The median life span of these two groups shortened progressively with rising P:C balance. For mated females, however, their median life span was the longest on the equally balanced (1:1) diet. The median numbers of eggs laid by mated females over 5 consecutive days were 1, 58 and 157 on 0:1, 1:1 and 4:1 diets, respectively (Kruskal–Wallis test: $H_2 = 51.58$, $P < 0.001$).

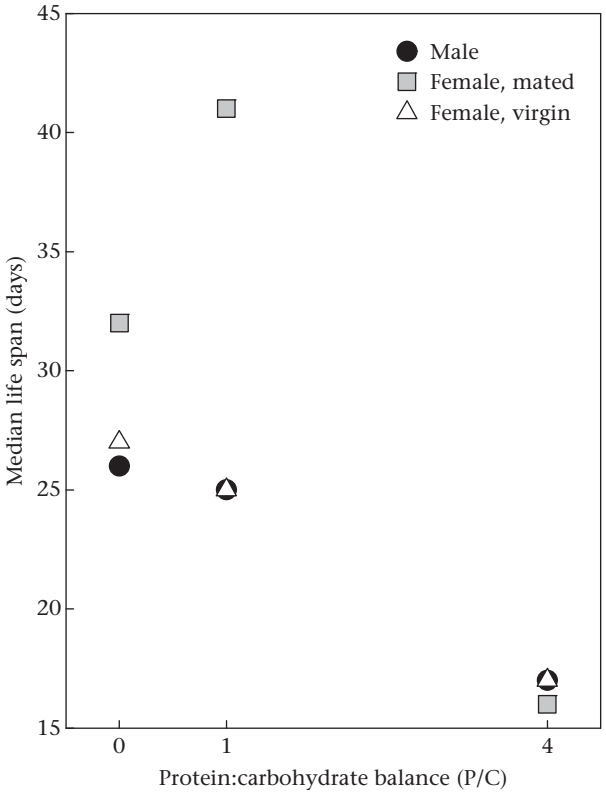

Figure 2. Median life span in relation to dietary P:C balance (0:1, 1:1 and 4:1) in male, mated female and virgin female *D. melanogaster*.

DISCUSSION

By employing chemically defined diets and quantifying actual nutrient intake, we were able to demonstrate dramatic effects of mating on the pattern of nutrient intake in female *D. melanogaster*. Our results showed that mated females consumed ca. 2.7 times more nutrients than both males and virgin females. This result is comparable to the ca. 2.3-fold increase in food intake that was previously observed from mated females of a different *D. melanogaster* strain (w1118) whose food intake was estimated using spectrophotometric analysis of ingested dyes (Carvalho et al. 2006). Mated females in our study ingested a consistently higher proportion of protein to carbohydrate than males and virgin females over the entire feeding period, and this finding is in close agreement with the recent studies that have demonstrated mating-induced preference for protein-rich yeast in female flies (Ribeiro & Dickson 2010; Vargas et al. 2010). Egg production requires protein (Wheeler 1996) so the increased egg production rate after mating is likely to induce high preference for protein-rich diets. Accordingly, our egg count data from the nutrient intake experiment indicated that egg production rate and protein preference were positively correlated. Recently, it has been demonstrated that a certain male seminal fluid protein called 'sex peptide (SP)' rapidly induces females to prefer protein-rich yeast by stimulating specific sensory neurons in the female's reproductive tract (Ribeiro & Dickson 2010). This SP-induced preference for protein-rich yeast is not driven by the depletion of the protein pool during egg development in females, indicating that the mechanism is 'nondemand mediated' (Barton Browne 1995; Simpson & Raubenheimer 2012). Additional 'demand-mediated' mechanisms, such as the neuronal target of the rapamycin (TOR)/S6 kinase signalling pathway and serotonin production in the central nervous system, are known to signal a fly's nutritional condition and to regulate how much protein is eaten (Ribeiro & Dickson 2010; Vargas et al. 2010; Simpson & Raubenheimer 2012). Whatever the mechanism, our results illustrate how profoundly the event of mating can affect feeding in this important model organism in both quantitative and qualitative ways.

Our results showed that mated females selected protein and carbohydrate in a 1:1.5 ratio, which was much higher than the ratio (1:4) that was recorded in an earlier study (Lee et al. 2008). The protein source of the synthetic diet used in the present study was sodium caseinate, a soluble milk-based protein, while that of the semisynthetic diet used in the earlier study was hydrolysed yeast, a composite ingredient that contains various other nutrients, including carbohydrates, lipids, minerals, etc. Amino acid compositions of these two sources of protein are likely to differ and it is possible that the observed disparity in the self-selected P:C ratio between the two studies is attributable to differences in amino acid compositions between the two protein sources. It has been demonstrated that some insects are capable of adjusting their preferred mixture of protein and carbohydrate intake based on the nutritional quality of proteins included in the diet (Lee 2007).

Our results showed that male flies self-composed a diet that contained four times more carbohydrate than protein (P:C 1:4). In *D. melanogaster* and other insects, males are expected to prefer carbohydrate-rich diets because male reproductive success depends on courtship activities and sexual traits that are energetically costly to express (Partridge et al. 1987; Maklakov et al. 2008; South et al. 2011). Throughout the feeding trial, male flies were confined singly in their own feeding vials in the absence of females. Hence, it

is possible that males might have self-composed even lower P:C ratios if they were given the opportunity to court and mate with females. However, a more striking result was the fact that despite sex differences in various features with respect to anatomy, physiology and behaviour, there was a remarkable similarity between males and virgin females in the amounts and mixture of ingested nutrients. These results suggest that mating is a significant factor influencing the expression of sexual dimorphism in nutrient intake and preference in *D. melanogaster*.

Life span is intimately linked to food intake in most organisms and the most well-known example of this relationship is the phenomenon referred to as dietary restriction, that is, life span extension through the moderate restriction of food intake. Traditionally, slow ageing caused by dietary restriction has been attributed to reduced energy intake (Weindruch & Walford 1988). However, mounting evidence from recent studies suggests that the dietary balance of specific nutrients (e.g. protein or amino acids, carbohydrate) is a key determinant of life span and ageing in *D. melanogaster* and other insects (Mair et al. 2005; Min & Tatar 2006; Lee et al. 2008; Maklakov et al. 2008; Fanson et al. 2009; Grandison et al. 2009a; Dussutour & Simpson 2012). Recent studies on dietary restriction in *D. melanogaster* have uniformly concluded that the overingestion of protein or amino acids shortens life span (Mair et al. 2005; Lee et al. 2008), but in these studies yeast was used as the main source of dietary protein. By using chemically defined diets, we have demonstrated that eating a diet containing excess protein (P:C 4:1) prompted flies to die sooner, confirming that the life-shortening effect of high yeast consumption was due to increased intake of protein rather than some unidentified components of yeast. Consistent with earlier results by Lee et al. (2008), egg production rate increased as diets contained more protein relative to carbohydrate. Reproduction imposes significant survival costs in many animals (reviewed by Harshman & Zera 2006; Flatt 2011). Therefore, it is necessary to investigate further whether the high mortality of females observed on this high-protein diet was the correlated response linked to elevated egg production rate or the direct result of lethal protein overingestion. One way to do so is to examine how the life span pattern responds to the same degree of protein overfeeding in males and virgin females, whose costs of egg production are expected to be either absent or minimal (Fanson et al. 2012). Our result clearly demonstrated that males and virgin females were susceptible to excessive ingestion of protein in their diet to a similar degree as mated females, providing strong support to the idea that protein ingestion in excess of requirement is responsible for shortening life span. The mechanisms of how excess protein intake shortens life span are not fully understood, but some emerging evidence indicates that the amino acid-sensitive, nutrient-sensing TOR pathway plays a pivotal role in regulating life span in *D. melanogaster* (Kapahi et al. 2004). Accumulation of toxic nitrogenous waste products derived from protein catabolism is also likely to reduce survival of flies on protein-rich diets.

We found a striking difference in life span between mated and virgin female *D. melanogaster* over the range of dietary P:C balance. As was the case for nutrient intake, the life span of virgin females was remarkably similar to that of males, but differed considerably from that of mated females. This indicates that sexual dimorphism in life span is not expressed constitutively, but is induced by mating in *D. melanogaster*. While the life span of males and virgin females tended to decline with rising dietary P:C balance, mated females had the longest life span on the 1:1 diet and the shortest on the 4:1 diet, with the life span on the 0:1 diet being intermediate. This difference in the life span–nutrient reaction norm between mated and virgin females indicates that the relationship between nutrient intake and life span is altered by mating in females, which is likely to be associated with changes in nutrient requirement and allocation induced by mating in female *D. melanogaster*.

While the pattern of high fertility and longer life span is normal in the reproductive female individuals of highly eusocial insects (e.g. queens of ants and honeybees; Remolina & Hughes 2008), reproduction is often negatively associated with life span in most animals, including *D. melanogaster* (Harshman & Zera 2006; Flatt 2011). Mating itself can reduce survival in female *D. melanogaster* through the effects of male seminal fluid proteins (Fowler & Partridge 1989; Chapman et al. 1995). In the present study, however, we observed that life span was prolonged for mated females in comparison to virgin females, most notably when they were fed a diet that contained an equal balance of protein and carbohydrate (P:C 1:1). The physiological basis underlying how life span was extended by mating is unclear, but it is possible that the postmating increase in food intake might have simultaneously allowed more resources to be allocated to somatic maintenance (survival) and to other physiological activities related to reproduction (van Noordwijk & de Jong 1986). In this experiment, female flies in the mating treatment were allowed to mate only for a limited period during their early adulthood (48 h just after emergence), suggesting that they did not suffer a significant life span reduction as a result of multiple mating throughout their lives (Chapman & Partridge 1996).

Our study highlights the importance of mating as a critical factor mediating sexual dimorphism in nutrient ingestion and life span in *D. melanogaster* and provides valuable insights into understanding the complex interconnections between sex, life span and nutrition.

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