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RESEARCH ARTICLE

Geometry of compensatory feeding and water consumption in *Drosophila melanogaster*

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SUMMARY

Feeding behaviour is an expression of an animal's underlying nutritional strategy. The study of feeding decisions can hence delineate nutritional strategies. Studies of *Drosophila melanogaster* feeding behaviour have yielded conflicting accounts, and little is known about how nutrients affect feeding patterns in this important model species. Here, we conducted two experiments to characterize nutrient prioritization and regulation. In a choice experiment, we allowed female flies to self-regulate their intake of yeast, sucrose and water by supplying individual flies with three microcapillary tubes: one containing only yeast of varying concentrations, another with just sucrose of varying concentrations, and the last with just water. Flies tightly regulated yeast and sucrose to a constant ratio at the expense of excess water intake, indicating that flies prioritize macronutrient regulation over excess water consumption. To determine the relative importance of yeast and sucrose, in a no-choice experiment, we provided flies with two microcapillary tubes: the first with one of the 28 diets varying in yeast and sucrose content and the other with only water. Flies increased total water intake in relation to yeast consumption but not sucrose consumption. Additionally, flies increased diet intake as diet concentration decreased and as the ratio of sugar to yeast equalized. Using a geometric scaling approach, we found that the patterns of diet intake can be explained by flies prioritizing protein and carbohydrates equally and by the lack of substitutability between the nutrients. We conclude by illustrating how our results harmonize conflicting results in the literature once viewed in a two-dimensional diet landscape.

Key words: dietary restriction, dehydration, Euclidean metric, fruit fly, geometric framework, macronutrient.

INTRODUCTION

Nutritional regulation entails a complex interplay between physiological and behavioural processes. Physiological needs are expressed through modulation of feeding behaviour, as animals strive to achieve a balance of nutrients that maximizes their individual fitness. This 'nutritional target' is often dynamic, changing with an animal's physiology and development stage (Raubenheimer and Simpson, 1999; Simpson and Raubenheimer, 1999; Simpson and Raubenheimer, 1993). When no single food provides a complete dietary solution but the environment contains a variety of foods that contain all the required dietary elements, animals can often regulate their intake of multiple nutrients, avoiding under- and over-ingestion of individual nutrients, and thereby attain their overall nutritional target (Lee et al., 2008; Maklakov et al., 2008; Raubenheimer and Simpson, 1993; Simpson et al., 2002). Assuming an evolutionary context, this precise regulation of nutrient intake reflects the fitness costs of non-optimal consumption of individual nutrients (Cheng et al., 2008; Lee et al., 2008; Simpson et al., 2004).

However, animals commonly live in variable and suboptimal environments and may sometimes be unable to reach their nutritional targets with the available food. For example, if a regulated nutrient is scarce and only found in foods also containing other nutrients, then to acquire the ideal intake of this nutrient animals would need to substantially over-consume other nutrients. Given there are fitness costs to suboptimal intake of each regulated nutrient class, the optimal intake strategy in a suboptimal environment is to minimize the total costs of under- and over-consumption of each nutrient (Cheng et al., 2008; Simpson et al., 2004). Thus, delineating the costs associated with under- and over-consumption of specific nutrients is key to understanding the regulation of feeding behaviour.

Assuming that intake is mediated by adaptive physiological processes that express the underlying fitness cost structure, observation of how animals regulate intake on imbalanced diets can reveal the costs associated with sub-optimal intake of different nutrients (Cheng et al., 2008; Raubenheimer and Simpson, 1999; Simpson and Raubenheimer, 1999). Measuring the diet intake of animals restricted to imbalanced diets and analysing the geometry of this regulated intake can illuminate the underlying cost structure (Cheng et al., 2008; Simpson et al., 2004). Using data from a variety of animals, Cheng and colleagues showed that these underlying cost structures are often quadratic (cost accelerating with deviation from the nutritional target) and that regulatory systems attempt to minimize the distance from current intake to the nutritional target in nutrient space, i.e. minimizing the Euclidean distance (Cheng et al., 2008). Analysis of these cost structures provides insight into the strategic basis of feeding behaviour.

Here, we utilized a geometric framework to better understand the role of nutrition in feeding behaviour in *Drosophila melanogaster* Meigen 1830 (hereafter *Drosophila*). Though commonly used in nutritional studies, surprisingly little is known about the relationship between nutrition and feeding behaviour in *Drosophila*. This is particularly evident in the conflicting results from studies of dietary restriction. Some studies have provided compelling evidence that

Drosophila are able to regulate nutrient intake. In particular, Lee and colleagues showed that *Drosophila* will strongly regulate sucrose and yeast consumption to a constant ratio when allowed to self-regulate (Lee et al., 2008). But conclusions from other studies have been inconsistent and even conflicting (Bross et al., 2005; Carvalho et al., 2005; Min and Tatar, 2006; Wong et al., 2009). The lack of consensus on whether or how *Drosophila* adjust feeding behaviour when restricted to sub-optimal diets presents an impediment to progress in this field (Flatt, 2011; Piper and Partridge, 2007). Given that at least some studies do provide support for intake regulation in *Drosophila*, we here sought to understand why others have not and thereby to reconcile the specific nutritional circumstances under which dietary regulation is evident.

In nutritional studies of *Drosophila*, diet quality is often manipulated by altering yeast, sugar and water content (Bross et al., 2005; Carvalho et al., 2005; Min and Tatar, 2006; Wong et al., 2009). The results of such studies are commonly interpreted in terms of yeast and sugar content, whereas water is usually viewed as a medium for dilution rather than as a distinct, and potentially regulated, element of diet. Yeast, sugar and water all have direct effects on fitness traits, especially lifespan and reproduction (Ja et al., 2009; Lee et al., 2008). Here, we first expand on the work of Lee and colleagues (Lee et al., 2008) by describing how *Drosophila* self-regulate yeast, sugar and water. Next, to elucidate the cost structure underlying yeast and sugar ingestion, we restricted flies to a systematic range of imbalanced diets. Sugar and water are distinct nutrient classes, but yeast is rich in diverse nutrient classes, including proteins, carbohydrates, minerals, sterols and vitamins. Of particular note, carbohydrates are available both as sucrose and as a component of yeast. Studies that have broken down food into particular nutrient classes have found that animals regulate feeding behaviour in terms of the protein and carbohydrate content within each food, rather than regulating the types of food (Cheng et al., 2008; Simpson and Raubenheimer, 1993; Simpson et al., 2004). Accordingly, we converted yeast and sugar into its protein and carbohydrate components to test whether these macronutrients better explain feeding patterns than food types.

MATERIALS AND METHODS

Husbandry

Drosophila melanogaster Canton-S were maintained at 25°C, 70% humidity on a 12 h/12 h light/dark cycle. Flies were cultured on a standard diet (85 g yeast, 125 g semolina, 125 g treacle, 12 g agar, 10 g nipagin in 1 litre of deionized water). Adult flies were collected within 24 h of emergence and fed the standard diet for 4 days to allow for mating. Females were then transferred by catching each fly in a 5 ml clear polystyrene vial that had three holes (1.2 mm in diameter) drilled into the bottom surface. These vials were inverted so that the cap formed the floor and the holes were on the upward-facing surface for insertion of microcapillary feeding tubes.

Experimental diets

We prepared 28 diets that varied in concentration (45, 90, 180, 360 g l^{-1}) and sucrose to yeast ratios (S:Y ratio: 1:0, 21:1, 3.4:1, 1.6:1, 1:1.5, 1:5, 0:1). Diets were prepared by dissolving hydrolysed yeast (MP Biomedicals, Aurora, OH, USA; no. 103304: 45% protein, 24% carbohydrate, 21% indigestible fibre, 8% water and 2% other) and sucrose (Sigma-Aldrich, St Louis, MO, USA; no. 84100) in deionized water. Food was supplied using a 5 µl microcapillary tube (Ja et al., 2007; Meats and Leighton, 2004).

Choice experiment

To characterize how *Drosophila* regulate water, yeast and sugar in concert, individual flies were provided with supplemental water and two separate microcapillary tubes containing only sucrose at 45, 90 or 180 g l^{-1} and only yeast at 45, 90 or 180 g l^{-1}. Microcapillary tubes were arranged in a straight line with the water in the middle position. We conducted two experimental runs with each diet combination using 6 flies (N=2 runs × 6 flies × 9 diets=108). Microcapillary tubes were replaced as needed to ensure flies always had diet accessible and any remaining liquid was measured using callipers. Diet and water consumption were measured for 4 days (described below).

No-choice experiment

We tested the effect of diet composition on water consumption. Flies were provided with supplemental water and one of 28 diets that varied in concentration and S:Y ratio. We conducted two experimental runs with each diet using 4 flies per run (N=2 runs × 4 flies × 28=224). Microcapillary tubes were replaced as needed. Diet and water consumption were measured for 6 days (described below).

Feeding assays

Depending on the experiment, each fly received either one or two microcapillary tubes containing diets and one microcapillary tube containing water. Individual containers were arranged on clear Plexiglas shelving units in a high humidity chamber (26±1.3°C, 85±3.6% humidity) and were photographed using a camera mounted at a fixed location every 20 min during daylight hours. Diets and water contained 0.4% blue food dye (Queen Fine Foods Pty Ltd, Alderly, QLD, Australia) to facilitate measurement. Photographs were corrected for barrel distortion using Adobe Photoshop CS4 (San Jose, CA, USA). Diet and water consumption were measured as the change in liquid displacement in the microcapillary tubes using ImageTool (v2.0 University of Texas Health Science Center, San Antonio, TX, USA). Correlation between the photographic method and measurement using callipers was r=0.987 (N=84).

To correct for evaporation, volume loss from each diet and for just water (two replicates per experimental run) was measured throughout the experiment in control vials that contained no flies. For these data, a regression model was conducted using solution concentrations to predict daily evaporative loss per 24 h. Using these predicted amounts, we then corrected for evaporation in vials containing flies using the following algorithm: (1) calculate the initial solution concentration for each day; (2) adjust daily consumption volume by subtracting predicted amount of water evaporation based on diet concentration; (3) calculate total diet (mg) consumed by multiplying initial concentration by adjusted consumption volume; (4) calculate new initial solution concentration for the following 24 h period by calculating total diet remaining in the microcapillary tube (initial diet – total consumed) and divide by the amount of liquid remaining.

Finally, to estimate water obtained from the diet, we fitted a surface regression model predicting the percentage of water in the diet in relation to sucrose and yeast concentrations. Using this model, we estimated water consumption by multiplying the diet consumed (adjusted for evaporation) by the predicted water percentage.

Data analyses

All statistical analyses were performed using SAS v9.1.3 (Cary, NC, USA). Parameter estimates from the models are presented as β ±

s.e.m. Flies that died were not included in the analyses. Protein and carbohydrate amounts were calculated using nutritional composition of yeast and sucrose.

Data from the choice experiment were analysed using a mixed linear model. For this analysis, we explored how the concentration of the yeast and sucrose solutions affected the consumed ratio of each pair of nutrients (W:Y, water:yeast ratio; W:S, water:sucrose ratio; S:Y, sucrose:yeast ratio). First, we determined the total amount of water, yeast and sucrose consumed over 4 days and used these values to calculate the three ratios for each individual fly. These ratios were then arctangent-transformed into radians. To account for the within-individual correlation among the three ratios, we modelled the covariance structure using a heterogeneous compound symmetry (Littell et al., 2006). This covariance matrix estimates separate variance for each response variable (ratio) and assumes all three response variables are equally correlated. For the fixed effects in the model, we included ratio type (W:Y, W:S, S:Y), yeast concentration and sucrose concentration, as well as all interactions to predict the consumed ratio (angle). In addition, we conducted a second-order surface analysis for the choice experiment in which supplemental water consumption was modelled in relation to yeast and sucrose concentrations. All models met normality and homoscedasticity assumptions given their covariance structure.

Data from the no-choice experiment were analysed using separate second-order surface analyses in which we included the main effects, their interaction, and the quadratic effects of each main effect (Myers et al., 2009). First, we explored the effects of yeast and sucrose concentrations on diet and supplemental water consumption over 6 days using separate models. Both response variables were centred, but no standardization was needed as variance and scaling were similar (Myers et al., 2009). Next, we explored total water intake in relation to actual sucrose and yeast consumption using separate surface models. Total water consumption was calculated by summing supplemental water and water from diet consumption. Finally, to better understand nutrient regulation from the no-choice experiment, we conducted a mixed linear model in which sucrose and yeast concentrations were used to predict total diet consumption (sucrose plus yeast) in relation to diet concentration and S:Y ratio. Total amounts were log-transformed to stabilize the variance. A similar analysis was then conducted using total nutrient consumption (carbohydrate and protein) in relation to diet dilution and C:P ratio. Again, total amounts were log-transformed to stabilize the variance. Following data transformations, all models met normality and homoscedasticity assumptions. As surface analyses are more readily visualized (Myers et al., 2009), we created surface plots from the fitted regression model.

Finally, to elucidate the cost structure of under- and over-consumption of diets, we modified a protocol outlined previously (Cheng et al., 2008) for estimating Euclidean cost structures for nutrient regulation patterns. We found the W (weighting factor) that minimized the Euclidean metric using least square means. In contrast to Cheng et al., who used the observed nutrient target to fix the curve in two-dimensional space, we allowed the algorithm to estimate the nutrient target but restricted the nutrient target to the observed 4:1 S:Y and C:P 9:1 trajectory from our choice experiment. This modification was employed because we did not have an equivalent nutrient intake for the high concentration from the choice experiment to match up with the high concentration of the no-choice experiment. Similar to Cheng et al., we calculated the error estimate by dividing the square root of the mean squared error by the distance of the nutrient target from the origin.

RESULTS

Choice experiment

The ratio analysis revealed only a ratio type × sucrose interaction ($F_{4,168}$=13.49, P<0.001); that is, the effect of sucrose concentration on diet ratio consumed depended on the ratio type (W:S, W:Y, S:Y). To better understand this interaction, we conducted orthogonal contrasts for each ratio type, testing for the effect of sucrose concentration. These contrasts revealed that sucrose concentration had an effect on W:S consumption ($F_{2,82.2}$=48.65, P<0.001), but no significant effect on S:Y consumption ($F_{2,94.5}$=0.9765, P=0.38) or W:Y consumption ($F_{2,92.7}$=2.36, P=0.10). Furthermore, sucrose concentration had a negative linear effect (linear trend contrast: $F_{1,82.2}$=93.86, P<0.001) on W:S consumption (Fig. 1A–C). Thus, increasing the concentration of sucrose in the microcapillary feeding tube decreased the W:S ratio of the overall diet consumed. To obtain estimates of the regulated ratios, we conducted linear contrasts, averaging yeast and sucrose concentrations for W:Y and S:Y consumption. The estimated back-transformed ratios from degrees were 88:1 (89.4±0.05 deg) for W:Y and 4:1 (76.8±1.1 deg) for S:Y. For W:S consumption we estimated ratios separately for each sucrose concentration because of the interaction; 28:1 (88.0±0.14 deg) at 45 g l^{-1}; 17:1 (86.7±0.14 deg) at 90 g l^{-1}; 14:1 (86.1±0.14 deg) at 180 g l^{-1}.

Flies increased supplemental water intake with increasing concentrations of sucrose [β_S=0.016±0.002 µl (g/l)$^{-1}$, P<0.001] and yeast [β_Y=0.007±0.002 µl (g/l)$^{-1}$, P=0.002], but no second-order effects were significant. Interestingly, flies consumed supplemental water on all yeast and sucrose combinations (Fig. 2). Even those on the 45 g l^{-1} sucrose and yeast diet combination still consumed an average of 1.3±0.24 µl supplemental water over 4 days.

No-choice experiment

Flies adjusted diet intake in relation to yeast and sucrose concentrations (Fig. 3A; Table 1). The highest overall intake was recorded in flies on more balanced diets in terms of yeast to sucrose ratio (Table 1; Fig. 3A). As diets became more imbalanced, overall diet intake decreased, especially for flies on sucrose-rich diets as the negative effect of sucrose concentration on consumption is three times that of yeast (Table 1; Fig. 3A). Converting the diet intake into yeast and sucrose amounts clearly shows that imbalanced diets (high sucrose or high yeast) had the lowest overall consumption of nutrients (Fig. 4A). On the highest yeast diets (S:Y 1:5 and 0:1), flies on the 180 and 360 g l^{-1} diets consumed similar amounts on each ratio (Fig. 4; t_{180}=0.46, P=0.64 and t_{180}= –1.07, P=0.29). Flies on the S:Y 1:0 diet had a striking reduction in total nutrient intake for all diet concentrations (Fig. 4A).

Diet concentration and S:Y ratios both affected supplemental water uptake. Increasing diet concentration resulted in flies consuming more supplemental water (Fig. 3B; Table 1). Additionally, changes in the concentration of yeast had an estimated threefold larger effect on supplemental water intake than did sucrose (Table 1). On the 45 g l^{-1} concentration, only flies on the S:Y 1:5 and 0:1 diets consumed supplemental water (1.19±0.29 and 1.28±0.29 µl, respectively). At the 90 g l^{-1} concentration, flies on all diets consumed supplemental water, except for those on the S:Y 1:0 and 21:1 diet, which did not consume significant volumes of water (0.16±0.12 and 0.21±0.12 µl, respectively).

The significant interaction between yeast and sucrose (Table 1) indicates that flies consumed less supplemental water when on balanced S:Y diets, and this is also evident in the convex shape of the consumption isoclines (Fig. 3B). This decrease in the consumption of supplemental water by flies on balanced diets is

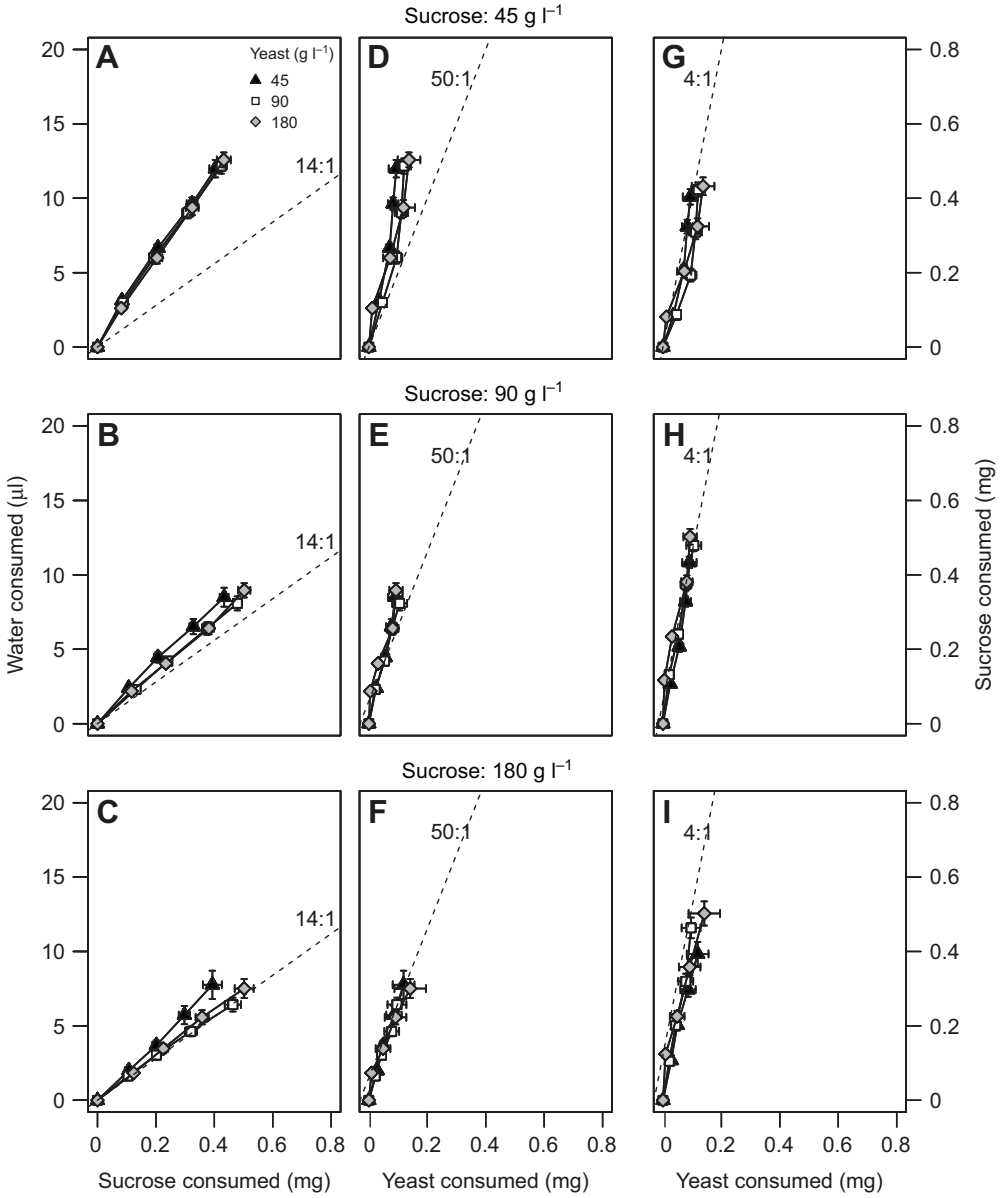

Fig. 1. Intake trajectories over 4 days for the choice experiment in which *Drosophila* were supplied with separate microcapillary tubes containing yeast only (key in A relates to all panels) and sucrose only (panels from top to bottom: 45, 90 and 180 g l^{-1}) of varying concentrations. Each point represents the daily mean cumulative consumption. The figures compare total water consumption in relation to sucrose consumption (A–C) and yeast consumption (D–F). The right panel compares sucrose consumption with yeast consumption (G–I). Dashed lines indicate estimated nutrient trajectories for flies supplied with high yeast (180 g l^{-1}) and sucrose (180 g l^{-1}) diets.

matched by increased diet consumption on these diets (Fig. 3A; Table 1). Summing supplemental water consumption and water from the diet revealed that flies consumed the most water when on diets containing S:Y of 1:1 to 1:5. Comparing total water intake to actual yeast and sucrose consumption shows a clearer picture. Yeast consumption significantly increased total water consumption (Fig. 5; β_Y=1.6±0.15 ln(µl) mg^{-1}, P<0.001, d.f.=202; $\beta_{Y\times Y}$=–0.49±0.08, P=0.001); whereas, sucrose had no significant effect (β_S=–0.01±0.2, P=0.94; $\beta_{S\times S}$=–0.12±0.12, P=0.32; $\beta_{S\times Y}$=0.10±0.13, P=0.43).

Modelling of Euclidean cost structures

For flies on the 360 g l^{-1} concentration of sucrose/yeast, total diet intake varied with S:Y composition (described above). How animals prioritize specific nutrients/food items can be characterized by assessing how much animals consume along different nutritional rails (Cheng et al., 2008; Raubenheimer and Simpson, 1993; Raubenheimer and Simpson, 1999; Simpson et al., 2004). The arc-shaped regulation pattern of intake for flies on the 360 g l^{-1} concentrations of the seven different S:Y ratios (Fig. 4) suggests a quadratic cost structure (Cheng et al., 2008; Simpson et al., 2004). Using sucrose and yeast consumption, we fitted a Euclidean metric; that is, we minimized the Euclidean distance between the nutrient target and intake along a nutritional rail (Fig. 6A). The fit for this curve was poor (residual error=0.37), especially for the lowest S:Y rails, suggesting that our model was inappropriate.

However, as some animals tightly regulate specific macronutrients rather than food items (Raubenheimer and Simpson, 1999; Simpson and Raubenheimer, 1993), we re-ran the curve fitting analysis using the macronutrient composition of the diets. For this analysis, we converted total yeast and sucrose consumed into protein and carbohydrate amounts (Fig. 4B). Again, we fitted cost curves using a Euclidean metric. The initial predicted curve fitted well for diets above C:P 9:1 (the target ratio), but fitted poorly for the carbohydrate-rich diets (C:P 47:1, 1:0). This result suggests that these points have a fundamentally different cost structure. Excluding C:P points greater than 9:1, the curve fit is very good (residual error=0.05 and a weighting factor of 1.2; Fig. 6B), indicating that these flies place a higher weight on protein regulation than carbohydrate regulation.

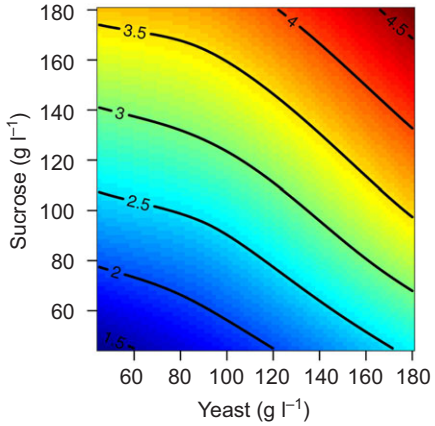

Fig. 2. Supplemental water intake (µl) for *Drosophila* from the choice experiment. Flies were supplied with three microcapillaries: yeast only, sucrose only and water. Axes represent the concentration of yeast and sucrose in the microcapillaries (45, 90 and 180 g l^{-1}) and the colours indicate the amount of water consumed over 4 days.

DISCUSSION

Water regulation

In diet manipulation studies, water is often treated as a matrix for nutrients rather than as a nutrient. However, our results clearly show that *Drosophila* do treat water as a nutrient and actively regulate water intake, especially in relation to yeast intake. However, flies prioritized yeast and sucrose consumption over excess water intake.

Prioritization of sucrose and yeast over water

When provided with separate sources of yeast, sucrose and supplemental water, *Drosophila* tightly regulated yeast and sucrose at a 4:1 S:Y ratio at the expense of excess water consumption. In order to achieve this S:Y ratio, flies on the lower sucrose concentration diet needed to employ compensatory feeding behaviour. As a result, flies on the lower sucrose concentration diet imbibed large quantities of dilute diet in order to meet their sucrose targets, and consequently consumed large quantities of water as a side effect. Flies supplied with the 45 g l^{-1} sucrose diet consumed almost double the total water intake of flies on the 180 g l^{-1} sucrose diet, but all diet combinations resulted in 4:1 S:Y intake. Assuming that intake priorities reflect the costs of over- and under-consumption, we can infer that the cost of water over-consumption is less than the cost of failing to achieve the preferred S:Y ratio. Lee and colleagues mapped lifetime egg production onto sucrose and yeast consumption and found significant fitness costs to deviations from the optimal ratio (Lee et al., 2008). However, little is known about the cost of excessive water consumption in *Drosophila*.

Water consumption in relation to yeast and sucrose

Drosophila consumed between 1.0 and 1.4 µl of water per day depending on the diet composition and concentration. This water intake is in line with water loss estimates of 0.8–2.0 µl day^{-1} for resting and hovering *Drosophila* (Lehmann et al., 2000). Activity for *Drosophila* in this study was mostly restricted to walking because of the container size and thus we would expect water loss patterns to be most similar to those of resting flies.

Interestingly, *Drosophila* increased total water intake in response to increased yeast consumption but not sucrose consumption. The most likely explanation for this pattern is that yeast contains significant levels of protein and salts, both of which increase overall osmolyte levels. The deamination of proteins in metabolism generates nitrogenous waste (e.g. uric acid) that must be excreted from the body, and this excretion can be a significant source of water loss (Karasov and Martinez del Rio, 2007). Furthermore, salts contribute directly to total osmotic load and excess salt must be excreted as well, which also results in water loss (Edney, 1977).

Water loss to egg production provides another potential explanation for the higher water needs of flies that consumed higher levels of yeast. Eggs require water and hence contribute to water loss in female insects (Edney, 1977). Water loss due to a single egg is small (0.008 µl egg^{-1}) (Limbourg and Zalokar, 1973), but *Drosophila* females can lay up to 100 eggs a day, resulting in significant water loss. As increases in yeast consumption are associated with increased egg production (Lee et al., 2008), female flies on high yeast diets should require more water. However, increased sucrose consumption at a constant level of yeast consumption also increases egg production (Lee et al., 2008), and flies did not increase water consumption in relation to sucrose consumption. This may be because flies are able to mitigate water loss from egg production through the generation of metabolic water by sucrose metabolism. Sucrose is a simple carbohydrate, does not produce nitrogenous waste requiring water for excretion, and only requires one water molecule to be catabolized into fructose and glucose, which can then be directly used in the metabolic pathway, resulting in the production of six molecules of water per sugar molecule.

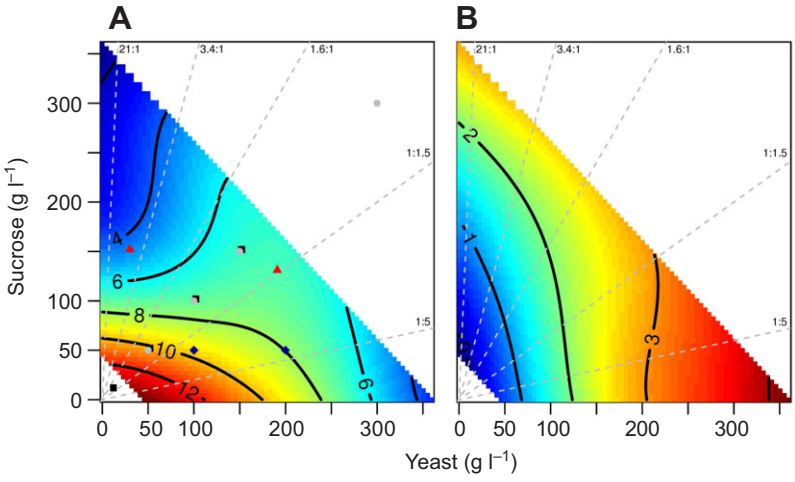

Fig. 3. Surface patterns in daily diet (A) and supplemental water (B) consumption (µl) in relation to yeast and sucrose concentrations of liquid diet from the no-choice experiment. Redder colours indicate higher diet/water consumption. Dashed grey lines show the sucrose:yeast (S:Y) nutritional rails used (no lines shown for 1:0 diet). Red triangles, black squares, grey circles and blue diamonds indicate the diet compositions used for measuring diet intake in Min and Tatar (Min and Tatar, 2006), Carvalho et al. (Carvalho et al., 2005), Bross et al. (Bross et al., 2005) and Wong et al. (Wong et al., 2009), respectively (see Discussion for more details).

Table 1. Parameter estimates for response surfaces predicting diet (μl), supplemental water (μl) and total water (μl) consumption over 6 days

	Diet		Supplemental water		Total water	
	Estimate ± s.e.m.	*P*-value	Estimate ± s.e.m.	*P*-value	Estimate ± s.e.m.	*P*-value
Sucrose	–0.10±0.01	<0.001	0.007±0.003	0.029	–0.43±0.33	0.19
Sucrose × sucrose	0.0002±0.00003	<0.001	0.00001±0.000008	0.35	0.13±0.20	0.49
Yeast	–0.03±0.01	0.009	0.021±0.003	<0.001	1.46±0.25	<0.001
Sucrose × yeast	0.0003±0.00005	<0.001	–0.00005±0.00002	0.002	0.19±0.19	0.33
Yeast × yeast	–0.00001±0.00003	0.64	–0.00003±0.000009	<0.001	–0.79±0.14	<0.001

For diet and supplemental water, estimates predict the relationship between consumption (μl) in relation to sucrose and yeast concentration (g l^{-1}) of the diets (see Fig. 3 for surface plots). For total water, estimates are based on amounts of sucrose and yeast consumed (mg) by flies (see Fig. 5 for surface plot).

Implications for other studies

Drosophila are routinely cultured without supplemental water (Bross et al., 2005; Hulbert et al., 2004; Libert et al., 2007; Skorupa et al., 2008), and hence researchers are implicitly assuming that *Drosophila* are able to acquire enough water from absorption of atmospheric water vapour, metabolic production of water and diet consumption (Edney, 1977). However, *Drosophila* have unusually rapid loss of water for its size (Hoffmann and Harshman, 1999), and metabolic water production only replenishes <10% of total water loss at rest and ~23% of water loss during flight (Lehmann et al., 2000). Furthermore, in *Drosophila pseudoobscura*, net water loss in adult flies is evident under all humidity conditions below saturation, suggesting there is little active absorption of water from the atmosphere (Arlian and Eckstrand, 1975). Ingestion of water hence appears to be necessary for the maintenance of proper water balance in these flies. Our results show that flies consumed supplemental water on all except the most dilute diets. Hence, flies maintained without supplemental water are likely to be water stressed.

Water stress has direct effects on fitness in *Drosophila*, and dietary treatments that induce water stress hence need to be interpreted with great caution. Ja and colleagues showed that *Drosophila* have reduced lifespan when on high concentration diets (200 g l^{-1}) compared with diluted diets (50 g l^{-1}) but this effect vanished when supplemental water was added to the containers (Ja et al., 2009). Hence the effects of diet on lifespan were probably due to water stress, and not to costs of reproduction as previously thought (Ja et al., 2009). Given the common experimental use of diet manipulations, confounding effects of water stress with diet quality could be widespread in the expression of a variety of traits, especially in cultured *Drosophila*, which lose desiccation resistance during the laboratory culturing process (Hoffmann et al., 2001). Our results provide a map of supplemental water intake for diets varying in sucrose and yeast concentration and hence facilitate estimation of the degree of water stress in previous studies.

Macronutrient regulation

Our results show that *Drosophila* have a complex compensatory feeding response to diet quality. The good fit of the Euclidean metric with a weighting factor of 1.2 suggests that this feeding pattern is due to the simultaneous regulation of protein and carbohydrates, with these nutrients having very similar priority and little substitutability. As shown below, mapping data from other studies onto our compensatory feeding surface unifies seemingly disparate results.

Geometry of protein and carbohydrate regulation

To understand the cost of diet components, we fitted a Euclidean metric, which minimizes the overall distance in two-dimensional space (Cheng et al., 2008). All curve fits were poor for sucrose and yeast consumption, with the predicted values deviating substantially for high and low S:Y rails. In contrast, the final curve fit using carbohydrate and protein consumption was very good. These curve fits provide several insights into nutrient regulation of these flies.

First, *Drosophila* regulate nutrition differently when on sugar-rich diets. Though the cost curve fits the intake for diets with C:P ratios below the nutrient target ratio, the two highest C:P rails had very poor fits. The fitted curve predicts much higher consumption on the high C:P diets than we obtained. *Drosophila* are physically capable of increasing their diet intake, as other C:P ratios had a much higher intake. Thus, *Drosophila* are actively restricting intake when on high C:P diets. It may be that flies are in a fundamentally different physiological state when on a low protein diet, as vitellogenesis ceases, leaving them in a reproductive diapause-like state (Good and Tatar, 2001). The nutritional requirement of flies

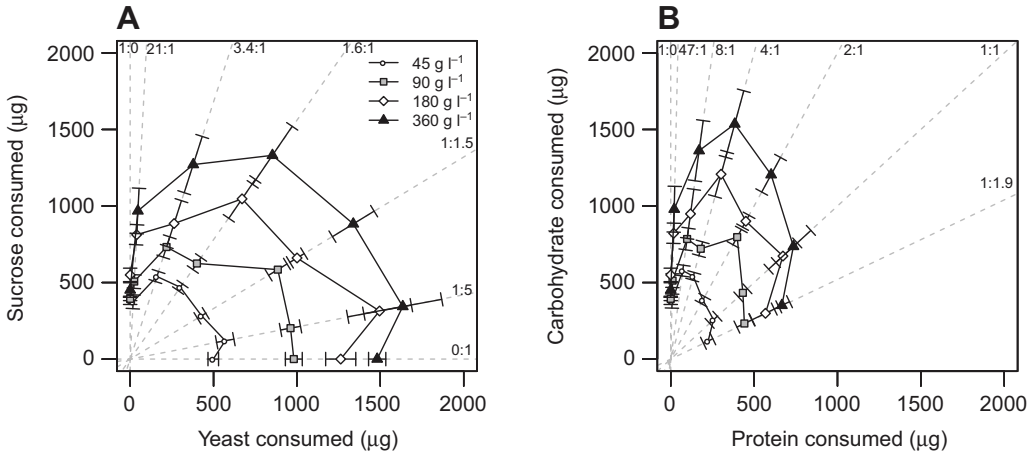

Fig. 4. Mean intake amounts for *Drosophila* on the different diets. (A) Sucrose and yeast consumption for the no-choice experiment. Different symbols represent different diet concentrations (see key). (B) Carbohydrate and protein consumption patterns. Dashed grey lines show S:Y (A) and carbohydrate:protein (C:P) (B) nutritional rails used. Error bars represent ±1 s.e.m.

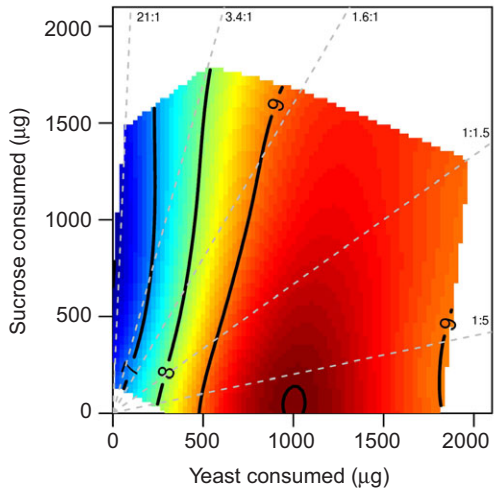

Fig. 5. Surface patterns of total water consumption (µl) in relation to actual amounts of sugar and yeast consumed. Values are total amounts over 6 days for *Drosophila*. Refer to Fig. 3 for details.

in this state may be fundamentally different and, concomitantly, the nutrient regulation strategy may also be different.

Second, for nutritional rails below C:P 9:1, *Drosophila* tightly regulate carbohydrate and protein intake following a quadratic cost structure. *Drosophila* regulated diets along different C:P rails, minimizing the Euclidean distance from the nutrient target to each C:P rail. This regulation pattern appears common in a variety of animals, from locust to chickens to humans (Cheng et al., 2008). The similar weighting of protein and carbohydrate by *Drosophila* may be the consequence of both nutrients having effects on key fitness traits. Lee and colleagues showed that *Drosophila* on low C:P rails had high egg production rates but short lifespans, whereas those on high C:P rails had long lifespans but low egg production rates (Lee al., 2008).

Third, the poor fit for S:Y intake compared with the good fit for C:P intake suggests that the other nutrients in yeast have less influence on intake patterns. While most often thought of as a source of protein, yeast is also rich in various micronutrients and macronutrients that are absent in sucrose. Consumption of these nutrients correlates perfectly with consumption of yeast, and hence the poor curve fit suggests that *Drosophila* treat these nutrients as lower priority. These results support the assertion of Lee and colleagues that *Drosophila* provided with diets of yeast and sucrose are principally regulating their intake of protein and carbohydrate (Lee et al., 2008). Furthermore, we (Fanson and Taylor, 2011) found that Queensland fruit flies (*Bactrocera tryoni*; Q-flies) fed on holidic diets varying only in amino acids and sucrose levels produced similar diet consumption patterns as flies on yeast and sucrose diets.

Finally, the curve fit suggests little substitutability between carbohydrates and protein. Protein can be deaminated and the carbon structure then used for energy metabolism, replacing carbohydrates as an energy source. This type of substitutability straightens the elliptical fit towards a straight line with a slope of –1, depending on the degree of substitutability (Cheng et al., 2008). Our final curve fit assumed no substitutability and fitted the data well. Adding substitutability into the metric only worsens the fit to the data.

Compensatory feeding in flies

Drosophila adjusted their volumetric intake in relation to both S:Y ratio and diet concentration. On a specific S:Y ratio, *Drosophila* exhibited compensatory feeding as diet concentration decreased, more than doubling intake on the lowest concentrations compared with the highest concentrations for several S:Y ratios. S:Y ratio also affected volumetric intake, with flies on high S:Y diets having lower diet intake. These plastic feeding patterns in *Drosophila* match those reported in Q-flies (Fanson et al., 2009).

The response surfaces of these feeding patterns resolve conflicting reports in the literature about compensatory feeding in *Drosophila*. Using radiotracers to measure diet intake, Carvalho and colleagues found strong support for compensatory feeding when diets of a constant S:Y were diluted from 300 to 20 g l^{-1} (Carvalho et al., 2005). These findings match well with our results (black squares in Fig. 3A). In another study, Bross and colleagues (Bross et al., 2005) measured dye uptake and found that male flies on the lowest concentration (S:Y 1:1, 100 g l^{-1}) had increased intake, but that flies at 200, 300 and 600 g l^{-1} had similar intake. We found a similar pattern, with flies on 100 g l^{-1} having a higher intake, whereas intake was similar for flies on 200 and 300 g l^{-1} (grey circles in Fig. 3A, we did not measure at 600 g l^{-1}). In a third study, using dye uptake and fecal pellets to measure feeding rate, Min and Tatar (Min and Tatar, 2006) found no evidence of compensatory feeding, as flies on a restricted diet (S:Y 5:1, 182 g l^{-1}) had a reduced feeding rate compared with those on a full diet (S:Y 1:1.45, 322 g l^{-1}). We found the same pattern in our results (red triangles in Fig. 3A), as the reduced intake due to switching to the higher S:Y of 5:1 outweighed the increased intake due to switching to the lower concentration.

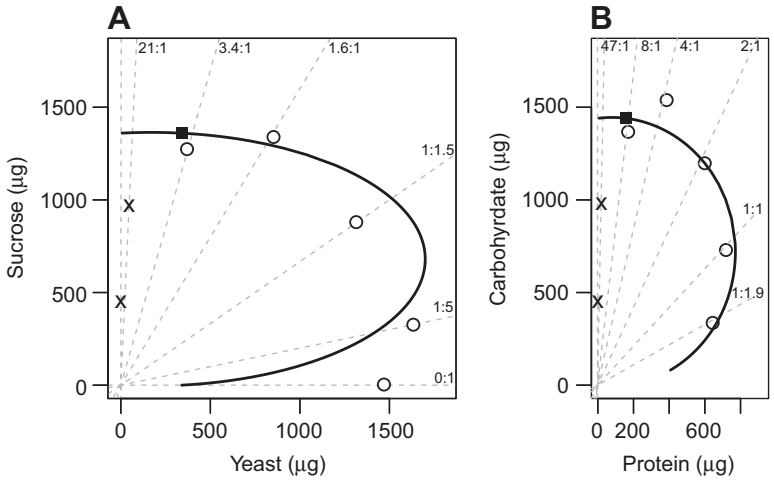

Fig. 6. Predicted nutrient regulation (black line) pattern based on Euclidean cost structure. The nutrient targets (black square) are based on self-selecting nutrient trajectories of S:Y 4:1 (A) and C:P 9:1 (B). Circles are the mean estimates from the no-choice experiment for flies on the 360 g l^{-1} diets. The two highest sugar diets (crosses) were excluded from the optimization algorithm (see Materials and methods and Results). Note – axes are scaled 1:1 between the plots.

Wong and colleagues (Wong et al., 2009) also found no evidence of compensatory feeding. They switched female flies from a full diet (S:Y 1:4, 250 g l^{-1}) to a restricted diet (S:Y 1:2, 150 g l^{-1}) and found no significant difference in the proportion of flies feeding between these two diets. Our surface regression derived from the measured intake of individual flies predicts only a modest increase in diet intake from 7.8±0.5 to 9.8±0.4 µl across this range, a change that we would not expect to be resolved by their indirect and comparatively coarse measure of proportion of 150 flies in a vial feeding. That is, rather than being inconsistent with our results, their study may be best interpreted as lacking the experimental power required to detect effects for the range of conditions investigated. Having harmonized the findings of our study with each of these previous findings, the only incongruent experimental result comes from the female flies studied by Bross and colleagues (Bross et al., 2005). In contrast to the smoothly progressive patterns found in the present study, they found inconsistent up-and-down intake patterns with increasing diet concentration as flies consumed more on the 100 and 600 g l^{-1} diets than on the 300 g l^{-1} diet.

Overall, our results clearly show why Carvalho and colleagues found compensatory feeding, Bross and colleagues found partial compensatory feeding, and Min and Tatar did not detect any evidence of compensatory feeding in *Drosophila*, and also indicate that, while not supporting compensatory feeding, the lack of evidence in the study by Wong and coworkers (Wong et al., 2009) should also not be taken as evidence against compensatory feeding. This reconciliation and integration of seemingly disparate data and conclusions into a common framework provides a stable base for the continued development of *Drosophila* as a model system for the study of nutrient regulation.

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REFERENCES

Arlian, L. G. and Eckstrand, I. A. (1975). Water balance in *Drosophila pseudoobscura*, and its ecological Implications. *Ann. Entomol. Soc. Am.* **68**, 827-832.

Bross, T. G., Rogina, B. and Helfand, S. L. (2005). Behavioral, physical, and demographic changes in *Drosophila* populations through dietary restriction. *Aging Cell* **4**, 309-317.

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

Cheng, K., Simpson, S. J. and Raubenheimer, D. (2008). A geometry of regulatory scaling. *Am. Nat.* **172**, 681-693.

Edney, E. B. (1977). *Water Balance in Land Arthropods*. New York: Springer-Verlag.

Fanson, B. G. and Taylor, P. W. (2011). Protein:carbohydrate ratios explain lifespan patterns found in Queensland fruit fly on diets varying in yeast:sugar ratios. *Age*, Epub ahead of print, PMID: 21904823.

Fanson, B. G., Weldon, C. W., Perez-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.

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

Good, T. P. and Tatar, M. (2001). Age-specific mortality and reproduction respond to adult dietary restriction in *Drosophila melanogaster*. *J. Insect Physiol.* **47**, 1467-1473.

Hoffmann, A. A. and Harshman, L. G. (1999). Desiccation and starvation resistance in *Drosophila*: patterns of variation at the species, population and intrapopulation levels. *Heredity* **83**, 637-643.

Hoffmann, A. A., Hallas, R., Sinclair, C. and Partridge, L. (2001). Rapid loss of stress resistance in *Drosophila melanogaster* under adaptation to laboratory culture. *Evolution* **55**, 436-438.

Hulbert, A. J., Clancy, D. J., Mair, W., Braeckman, B. P., Gems, D. and Partridge, L. (2004). Metabolic rate is not reduced by dietary-restriction or by lowered insulin/IGF-1 signalling and is not correlated with individual lifespan in *Drosophila melanogaster*. *Exp. Gerontol.* **39**, 1137-1143.

Ja, W. W., Carvalho, G. B., Mak, E. M., de la Rosa, N. N., Fang, A. Y., Liong, J. C., Brummel, T. and Benzer, S. (2007). Prandiology of *Drosophila* and the CAFE assay. *Proc. Natl. Acad. Sci. USA* **104**, 8253-8256.

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. Natl. Acad. Sci. USA* **106**, 18633-18637.

Karasov, W. and Martinez del Rio, C. (2007). *Physiological Ecology: How Animals Process Energy, Nutrients, and Toxins*. Princeton, NJ: Princeton University Press.

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. Natl. Acad. Sci. USA* **105**, 2498-2503.

Lehmann, F. O., Dickinson, M. H. and Staunton, J. (2000). The scaling of carbon dioxide release and respiratory water loss in flying fruit flies (*Drosophila* spp.). *J. Exp. Biol.* **203**, 1613-1624.

Libert, S., Zwiener, J., Chu, X., VanVoorhies, W., Roman, G. and Pletcher, S. D. (2007). Regulation of *Drosophila* life span by olfaction and food-derived odors. *Science* **315**, 1133-1137.

Limbourg, B. and Zalokar, M. (1973). Permeabilization of *Drosophila* eggs. *Dev. Biol.* **35**, 382-387.

Littell, R. C., Milliken, G. A., Stroup, W. W., Wolfinger, R. D. and Schabenberger, O. (2006). *SAS System for Mixed Models*. Cary, NC: SAS Institute Inc.

Maklakov, A. A., Simpson, S. J., Zajitschek, F., Hall, M. D., Dessmann, J., Clissold, F., 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.

Meats, A. and Leighton, S. M. (2004). Protein consumption by mated, unmated, sterile and fertile adults of the Queensland fruit fly, *Bactrocera tryoni*, and its relation to egg production. *Physiol. Entomol.* **29**, 176-182.

Min, K. J. and Tatar, M. (2006). *Drosophila* diet restriction in practice: do flies consume fewer nutrients? *Mech. Ageing Dev.* **127**, 93-96.

Myers, R. H., Montgomery, D. C. and Anderson-Cook, C. M. (2009). *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*. Hoboken, NJ: Wiley.

Piper, M. D. W. and Partridge, L. (2007). Dietary restriction in *Drosophila*: delayed aging or experimental artefact? *PLoS Genet.* **3**, e57.

Raubenheimer, D. and Simpson, S. J. (1993). The geometry of compensatory feeding in the locust. *Anim. Behav.* **45**, 953-964.

Raubenheimer, D. and Simpson, S. J. (1999). Integrating nutrition: a geometrical approach. *Entomol. Exp. Appl.* **91**, 67-82.

Simpson, S. J. and Raubenheimer, D. (1993). A multilevel analysis of feeding behavior-the geometry of nutritional decisions. *Philos. Trans. R. Soc. Lond. B* **342**, 381-402.

Simpson, S. J. and Raubenheimer, D. (1999). Assuaging nutritional complexity, a geometrical approach. *Proc. Nutr. Soc.* **58**, 779-789.

Simpson, S. J., Raubenheimer, D., Behmer, S. T., Whitworth, A. and Wright, G. A. (2002). A comparison of nutritional regulation in solitarious- and gregarious-phase nymphs of the desert locust *Schistocerca gregaria*. *J. Exp. Biol.* **205**, 121-129.

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.

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.

Wong, R., Piper, M. D. W., Wertheim, B. and Partridge, L. (2009). Quantification of food intake in *Drosophila*. *PLoS ONE* **4**, e6063.