

Sex-Specific Fitness Consequences of Nutrient Intake and the Evolvability of Diet Preferences

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Submitted August 27, 2012; Accepted February 18, 2013; Electronically published May 22, 2013

Online enhancement: appendix. Dryad data: <http://dx.doi.org/10.5061/dryad.55724>.

ABSTRACT: The acquisition of nutrients is fundamental for the maintenance of bodily functions, growth, and reproduction in animals. As a result, fitness can be maximized only when animals are able to direct their attention to foods that reflect their current nutritional needs. Despite significant literature documenting the fitness consequences of nutrient composition and preference, less is known about the underlying genetic architecture of the dietary preferences themselves, specifically, the degree to which they can respond to selection. We addressed this by integrating evolutionary quantitative genetics and nutritional geometry to examine the shape of the sex-specific fitness surfaces and the availability of genetic variance for macronutrient preferences in the fruit fly *Drosophila melanogaster*. Combining these analyses, we found that the microevolutionary potential of carbohydrate and protein preference was above average in this population, because the expected direction of selection was relatively well aligned with the major axis of the genetic variance-covariance matrix, **G**. We also found that potential exists for sexually antagonistic genetic constraint in this system; macronutrient blends maximizing fitness differed between the sexes, and cross-sex genetic correlations for their consumption were positive. However, both sexes were displaced from their feeding optima, generating similar directional selection on males and females, with the combined effect being that minimal sex-specific genetic constraints currently affect dietary preferences in this population.

Keywords: quantitative genetics, nutritional geometry, feeding preference, genetic constraint, *Drosophila melanogaster*, DGRP.

Introduction

The acquisition of energy through the consumption of nutrients is a fundamental requirement for all living organisms. Food intake affects fitness not only by way of the total amount of energy acquired but also through the specific composition of macronutrients consumed (Rauben-

heimer et al. 2009; Simpson et al. 2010; Ward et al. 2011). The nutritional composition of an individual's diet, commonly expressed as the consumption of the three major macronutrients (protein, carbohydrate, and fat) during the adult/reproductive phase, can affect a wide range of fitness components, including life span (Ja et al. 2007; Lee et al. 2008; Grandison et al. 2009; Piper et al. 2011; Nakagawa et al. 2012), fecundity (Lee et al. 2008; Maklakov et al. 2008), secondary sexual trait expression (Hunt et al. 2004; Maklakov et al. 2008; South et al. 2011), and success in postcopulatory sexual selection (Fricke et al. 2008). Furthermore, if the sexes maximize fitness in different ways, then the nutritional requirements may also be sex specific (Maklakov et al. 2008). To ingest an optimal composition of macronutrients, an organism needs to first assess its current nutritional needs and then feed in a manner that reflects those needs. In this way, feeding involves both a time investment and, critically, the expression of a preference for specific combinations of nutrients (Behmer 2009; Raubenheimer et al. 2012).

Dietary preferences are expected to play an important role in determining an animal's nutrient intake because they enable an organism to regulate intake from multiple food sources and compensate for specific nutrients that might be limited in availability (Edgecomb et al. 1994; Raubenheimer and Jones 2006; Behmer and Joern 2008; Sørensen et al. 2008). The consumption of different macronutrients should result in a diet that, subject to availability, maintains health and maximizes fitness (Behmer 2009; Raubenheimer et al. 2009; Simpson et al. 2010; Hewson-Hughes et al. 2011). This has been exemplified in studies of female *Drosophila melanogaster* (Lee et al. 2008) and male cockroaches *Nauphoeta cinerea* (South et al. 2011), where the preferred blend of nutrients reflected the blend that maximized the fitness component measured. However, despite a growing understanding of the fitness consequences of nutrient composition (Lee et al. 2008; Maklakov et al. 2008; Fanson et al. 2009; Fanson

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and Taylor 2011; South et al. 2011) and adaptation to experimental changes in diet (Rundle et al. 2006; Warbrick-Smith et al. 2006, 2009; Kolss et al. 2009; Vijendra-varma et al. 2011; Attisano et al. 2012), our understanding of the genetic architecture of dietary preferences remains limited. The nature of any genetic variation in diet preferences may have important consequences for a population's ability to adapt to multigenerational changes in nutrient availability (Warbrick-Smith et al. 2009; Raubenheimer et al. 2012) and may determine the extent to which sexually dimorphic preferences that match sex-specific dietary optima can evolve (Maklakov et al. 2008).

Using the model species *D. melanogaster*, we set out to determine (1) how adult consumption of protein and carbohydrate influences sex-specific fitness and (2) the amount of standing genetic variance available for adult dietary preferences to respond to sex-specific selection. Although previous studies have examined effects of diet composition on fitness components in *D. melanogaster* (Fricke et al. 2008; Lee et al. 2008), this relationship has yet to be simultaneously quantified for both sexes in this species. In order to address the empirical gap between the fitness effects of diet and the genetic basis of dietary preferences, we integrated evolutionary quantitative genetic and nutritional geometry approaches. Nutritional geometry involves the construction of a specific number of diets that vary in both the concentration and the ratio of the macronutrients of interest (Raubenheimer and Simpson 1997). Within this framework, the effect of the intake of specific nutrient combinations on other phenotypic measurements, such as components of fitness, can be estimated. The data collected can be used to estimate response surfaces for nutritional composition through the application of classic approaches to the measurement of selection (Lande and Arnold 1983). Second, by conducting preference trials where individuals choose between diets composed of specific macronutrients incorporated within a quantitative genetic breeding design, it is possible to predict the potential for dietary preferences to adapt from standing variation. By combining these two approaches, we estimated the evolvability—the availability of genetic variance along estimated vectors of optimal selective response—of macronutrient preferences within an estimated fitness response surface, giving insight into the potential for genetic constraints to affect their evolution (Hansen and Houle 2008).

Methods

All experiments were conducted using lines of *Drosophila melanogaster* that are a randomly selected subset of the *Drosophila* genetic reference panel (DGRP) initially established by T. Mackay, North Carolina State University

(Mackay et al. 2012), and were sourced from the Bloomington Stock Center. A list of lines used in each experiment is provided in table A1 (tables A1–A3 are available online). We maintained lines on a standard fly medium containing sugar, yeast, and polenta mixed in an agar solution. Flies were kept in a temperature-controlled room at 25°C and a 12L : 12D cycle. Brown-eyed mutant flies used in the competitive fitness assays were maintained under the same rearing protocol.

Experiment 1: Estimating the Fitness Surface for Adult Diet

We used a no-choice design to test for sex-specific dietary fitness optima. Larvae were density controlled one generation before the experiment. Virgin males and females were sexed from the emerging larvae using a light CO₂ anesthesia and kept individually in glass vials containing 5 mL of agar solution, which stops desiccation of the flies but contains no calories. The flies were held overnight on this medium before being supplied with a 5- μ L microcapillary tube (Drummond Microcaps) containing one of 24 different protein : carbohydrate (P : C) diets, allowing for real-time measurements of the macronutrients ingested by single flies (Ja et al. 2007). Nutrient availability was manipulated using the geometric framework (Raubenheimer and Simpson 1997), where P : C ratios and dilution levels were altered, producing a range of different diets. There were six different protein to carbohydrate ratios (1 : 16, 1 : 8, 1 : 4, 1 : 2, 1 : 1, and 1.27 : 1) and four levels of concentration (9, 18, 27, and 36 g of solute per 100 mL of solution). The six different P : C ratios were generated by varying the amount of hydrolyzed yeast (MP Biomedicals, catalog 103304) to sucrose in the following ratios: 1 : 7, 1 : 3.4, 1 : 1.6, 1 : 0.7, and 1 : 0.1 (Lee et al. 2008). To estimate the population-level response surface, a total of 960 (480 male and 480 female) virgin flies, taken equally from 37 randomly selected DGRP lines (table A1), were randomly allocated to the 24 diets. Our goal here was not to estimate line-level fitness optima but rather to obtain an overall population estimate.

Flies were exposed to the diet treatments over four consecutive days in a climate simulator (Contherm Scientific) at a constant temperature (25°C), light cycle (12L : 12D), and high relative humidity (85%–90%). The microcapillary tubes were replaced for all diets on day 2. The rate of evaporation for each diet was measured using five vials per diet that contained no flies, placed randomly within the climate simulator. Competitor flies that were homozygous for a recessive brown eye color mutation were also sexed as virgins and held five per vial on standard fly media, along with access to live yeast ad lib., at 25°C with a 12L : 12D cycle concurrently with the experiment.

We used a competitive adult fitness assay to estimate male and female fitness in the different diets. A focal fly is put into a vial with male and female flies homozygous for a recessive eye color mutation, and the flies are left to compete for mating opportunities; since the mutation is recessive, it is possible to determine paternity/maternity of the focal and competitor flies in the emerging progeny. This approach has successfully been used as an assay for adult fitness in several previous studies (Chippindale et al. 2001; Delcourt et al. 2009). On day 4 of the feeding trial, flies of both sexes were transferred to a new vial containing 10 mL of standard fly media simultaneously with a brown-eyed (*br/br*) competitor male and female fly, so that each vial in our competitive trial contained three flies: a focal DGRP sourced fly from the diet treatment, a *br/br* male, and a *br/br* female. Although the introduction of our focal flies to standard fly media would allow them access to a limited food source, it is important to note that these vials were void of live yeast, which is the main source of nutrition for laboratory-reared adult *Drosophila* (Sang 1978). In *D. melanogaster*, flies with limited/no access to live yeast show reduced fecundity in females (Stewart et al. 2005) and reduced paternity in males (Fricke et al. 2008). We set up a total of 835 competition vials with the aim of determining the influence of nutrient consumption on competitive fitness; flies that died during the feeding trials ($n = 125$) were unable to be included in the analysis of this component of fitness. In the competitive fitness trials, the flies were allowed to interact for 24 hours, after which all flies were removed and the vials were retained for offspring development. On days 10 and 11, all emerged adults were counted and scored for the presence or absence of the recessive eye mutation, hence determining paternity/maternity.

Data Analysis

Consumption for individual flies was estimated by subtracting the amount of food remaining from the total length of the 5 μ L microcapillary tube, adjusting for evaporation rate of a particular diet. These volumes were then converted into total micrograms of protein and carbohydrate consumed by each fly. Adult competitive fitness was calculated as the log odds, the natural log of the number of offspring produced by the focal fly in a fitness assay (*wt*) divided by the offspring produced by the competitor fly (*br*) (Chippindale et al. 2001; Delcourt et al. 2009):

$$\omega = \ln\left(\frac{wt + 1}{br + 1}\right). \quad (1)$$

A value of 1 was added to both the numerator and the denominator to avoid attempting to take the natural log

of any zero scores in the data set (eq. [1]). Competition vials that produced no offspring (neither red or brown eyed) were removed from the data set ($n = 35$ of 835 trials), since the focal flies' failure to produce offspring is unlikely to have resulted from the competitive interaction within the vial. However, including these data did not change the reported findings (results not shown).

We estimated the effect of protein and carbohydrate consumption on fitness in each of the sexes using a response surface analysis, implemented with the RSREG procedure in SAS (ver. 9.3; SAS Institute, Cary, NC). This approach analyzes individual fitness as a second-order polynomial function of the consumption of protein and carbohydrate via the following linear model:

$$\omega = \alpha_0 + \beta_1 P + \beta_2 C + \gamma_1 P^2 + \gamma_2 C^2 + \gamma_3 CP + \varepsilon, \quad (2)$$

where fitness ω , as measured in equation (1), is modeled as a function of the effects of the consumption of protein (P) and carbohydrate (C), the quadratic effects of protein (P^2) and carbohydrate (C^2), and the protein and carbohydrate cross product (CP). The overall significance of the model is tested using type I sums of squares. This model is essentially the same as that fitted to estimate standard nonlinear selection gradients in evolutionary quantitative genetics (Lande and Arnold 1983). However, its interpretation differs in our case because the variation in protein and carbohydrate concentrations is experimentally fixed rather than randomly sampled from a population. The linear and nonlinear estimates from this experimental design provide details of the fitness surface but are not selection gradients per se. Importantly, the estimates represent a phenotypic manipulation that allows robust estimation of areas of the fitness surface that may fall at the limits of the typical phenotypic range of the study population (Calsbeek et al. 2012). An inference of selection requires estimation of the population mean protein and carbohydrate consumption. To obtain these estimates and infer possible selection, we used data from a second experiment (experiment 2) in combination with the surface estimated from experiment 1 (full details are provided in "Data Analysis" for experiment 2).

To assess the shape of the estimated fitness surface of each sex, a γ matrix was constructed from the cross product and the quadratic components (Lande and Arnold 1983) in equation (2). We performed a canonical analysis of the γ matrix to derive the independent axes of bivariate food consumption along which curvature of the fitness surface was maximized (Phillips and Arnold 1989; Blows and Brooks 2003).

We tested the significance of each eigenvalue of γ (H_0 ; $\lambda_i = 0$ and no significantly detectable curvature along the corresponding eigenvector), using a randomization approach developed by Reynolds et al. (2010) and imple-

mented in R (ver. 2.15.0; code available in Reynolds et al. 2010). To test for sex differences in the shape of the linear and nonlinear fitness surface, we employed the procedure outlined by Chenoweth and Blows (2005). Briefly, a reduced model containing the linear, quadratic, and cross product component terms of the quantitative (nutrient consumption) variables and the qualitative (sex) variable is compared with the full model, which includes the addition of the interaction terms between sex and all quantitative components. A likelihood ratio test (LRT; $df = 3$) was used to test whether the inclusion of the trait \times sex interactions (two quadratic plus a correlational term) significantly improved the fit of the model. Models were run using SAS with maximum likelihood (ML) parameter estimation.

Experiment 2: Genetic Variance and Evolvability of Dietary Preference

To estimate genetic variance for and genetic covariances between components of diet preference, we used a binary choice assay in which flies were given unrestricted access to the two different macronutrients. Larval density was controlled one generation before the experiment, as in experiment 1, and the resulting progeny were sexed as virgins across three replicate vials per line. Flies were held individually overnight on 5 mL of agar solution, as in experiment 1. We sexed individual flies (940 flies) from across 48 lines, which included 36 lines from experiment 1, plus the addition of a further 12 randomly selected DGRP lines (line identification numbers are provided in table A1). The flies were each provided two feeding microcapillaries, placed into individual vials, and left to feed for 4 days under the same conditions and in the same constant temperature cabinet as in experiment 1. In each vial, one microcapillary contained a yeast solution, while the other contained a sucrose solution, both mixed at 30 g per 100 mL. To control for differences in the mean evaporation rate for the different macronutrients, 27 vials that contained no flies were placed randomly within the climate simulator. Consumption from each microcapillary was converted to amount of protein and carbohydrates consumed. Although the yeast solution used in the preference test is primarily protein (45%), it is not possible to remove all digestible carbohydrates (24%). Inclusion of the carbohydrate consumption from the yeast solution microcapillary in the genetic analyses would have introduced autocorrelation between carbohydrate and protein, thereby inflating any covariance between them. We therefore excluded carbohydrates potentially acquired from the yeast solution, making our overall estimates of carbohydrate consumption in experiment 2 slightly more conservative. Nevertheless, within these preference trials, the flies consumed far more of the sucrose solution than the

protein solution; thus, our decision to exclude carbohydrates procured from the yeast solution will have only a negligible influence on the estimated population mean carbohydrate consumption (a drop of 2.4% in females and 1.7% in males).

Data Analysis

We tested for genetic variance for dietary preferences with a multivariate linear mixed-effects model fitted using the Mixed procedure in SAS. To estimate the cross-sex genetic covariances for the macronutrients (carbohydrate, protein), each nutrient-sex combination was treated as a separate trait, resulting in four instead of two traits in the analysis. The following multivariate mixed-effects model was fitted to the data using restricted maximum likelihood (REML):

$$y = \mu + l + v(l) + \varepsilon, \quad (3)$$

where l is the random effect of line, v is the random effect of vial nested within line, and ε is the unexplained error. We compared differences in $-2 \log$ likelihood between a model run with and without the line term included and used LRTs to compare whether removal of the line term significantly worsened the fit of the model. The resulting (broad-sense) genetic variance-covariance (\mathbf{G}) matrix due to variation among lines can be partitioned into four submatrices, following Lande (1980):

$$\mathbf{G} = \begin{bmatrix} \mathbf{G}_m & \mathbf{B} \\ \mathbf{B}^T & \mathbf{G}_f \end{bmatrix}, \quad (4)$$

where \mathbf{G}_m and \mathbf{G}_f are the within-sex variance-covariance matrices, while \mathbf{B} and its transpose, \mathbf{B}^T , are the between-sex covariance matrices that are the ultimate determinants of responses due to indirect selection between the sexes.

To predict the ability of dietary preferences to respond to selection, we first determined the direction of optimal response using the RIDGE function implemented with the RSREG procedure in SAS. The RIDGE function allows the estimation—from user-specified starting coordinates—of a vector of optimal response within a known response surface. The starting coordinates in our case were the male and female mean consumption values for protein and carbohydrate from experiment 2, corrected for evaporation. We chose to estimate the vector of optimal response, β , in this manner rather than the linear estimates from the regression analysis in experiment 1, since our predictor variables from these fitness assays were experimentally fixed (see “Experiment 1: Estimating the Fitness Surface for Adult Diet”). The estimates of linear partial regression coefficients using data from experiment 1 reflect deviations

from a mean that is a property of our experimental response surface design rather than representative of a specific population, whereas the ridge analysis enabled us to estimate the direction of a maximal increase in fitness from the appropriate population means, determined from experiment 2.

To estimate population-level evolvability for male and female dietary preferences, we applied a framework developed by Hansen and Houle (2008). Briefly, this approach is derived from the Lande equation, $\Delta z = \mathbf{G}\beta$ (Lande 1979), and describes the multivariate evolutionary potential along a vector of optimal response, β , while accounting for the available genetic variance described by the genetic variance-covariance matrix, \mathbf{G} , estimated in equation (3). Using this approach, we calculated the unconditional evolvability, $e(\beta)$, which describes the length of the vector of the predicted response, Δz , within the space of \mathbf{G} projected onto the normalized vector of optimal response, β :

$$e(\beta) = \frac{[\beta^T][\mathbf{G}][\beta]}{|\beta|^2}, \quad (5)$$

where T denotes matrix transposition and $|\beta|$ is the length of β . We used the estimated vector of optimal response from the ridge analysis as β and complemented our sex-combined approach in the estimation of \mathbf{G} by combining our estimated β vectors for males and females, so

$$\beta = \begin{bmatrix} \beta_m \\ \beta_f \end{bmatrix}. \quad (6)$$

To allow for a meaningful interpretation of our estimation of $e(\beta)$, we estimated the average evolvability of \mathbf{G} :

$$\bar{e} = \frac{\sum_i \lambda_i}{k}, \quad (7)$$

where λ_i are the eigenvalues of \mathbf{G} and k equals the total number of eigenvalues (Hansen and Houle 2008). To estimate the sampling variance of our estimates of $e(\beta)$, we reestimated \mathbf{G} fitting model (2) using MCMCglmm (Hadfield 2010) in R (ver. 2.15.0), and we used the posterior distribution of the variance and covariance components of \mathbf{G} to calculate the posterior distributions of our evolvability metrics. Specifically, to assess the potential for dietary preferences to evolve in the direction of β , we estimated posteriors for the differences between $e(\beta)$ and the average evolvability, \bar{e} , of \mathbf{G} as well as its maximum e_{\max} and minimum e_{\min} (equal to largest and smallest eigenvalues of \mathbf{G}). We ran MCMCglmm for 100,000 iterations, with a burn-in of 20,000 iterations, and used parameter-expanded priors. Convergence of runs was con-

firmed by visually inspecting output plots, as per Hadfield (2012).

Results

The Fitness Effects of Adult Diet

In experiment 1, we found adult diet to be a significant linear predictor of variation in competitive fitness in *Drosophila melanogaster* males ($F_{2,420} = 4.55$, $P = .011$, $R^2_{\text{adj}} = 0.017$) and females ($F_{5,378} = 15.97$, $P \leq .001$, $R^2_{\text{adj}} = 0.073$). Of the two macronutrients, only carbohydrate consumption showed evidence for a linear relationship with fitness in males and females (table 1). We found no statistical evidence for differences in the slopes between the sexes using our sequential model building approach (LRT; difference $-2\ln L = 1.1$, $df = 2$, $P = .577$). For the nonlinear components, we again found that adult diet explained a significant proportion of both male ($F_{5,420} = 4.12$, $P \leq .001$, $R^2 = 0.047$) and female ($F_{5,378} = 8.10$, $P \leq .001$, $R^2 = 0.098$) competitive fitness. Our response surface analysis revealed that the protein-carbohydrate cross product significantly affected both male and female fitness (table 1). However, only male competitive fitness was significantly influenced by the consumption of protein in a nonlinear fashion. The negative coefficient indicates that the male response surface was convex in shape for protein intake, so that extreme low and high values for male protein consumption bestow lower fitness compared with intermediate consumption of protein (table 1). Unlike the linear component, our sequential model building approach showed significant sex differences in the nonlinear components of the fitness surfaces (LRT; difference $-2\ln L = 13.8$, $df = 5$, $P = .017$; fig. 1). The coefficients from the full model showed that males and females differed in the quadratic component of protein ($F_{1,788} = 6.00$, $P = .015$; table 1) and the protein-carbohydrate cross product ($F_{1,788} = 6.29$, $P = .0124$; table 1).

The overall nonlinear shape of the response surfaces that could be statistically supported resembles convex (stabilizing) selection. Canonical analysis of the γ matrices for males and females indicated that the response surfaces were saddle shaped, meaning that each had a concave (disruptive) and convex (stabilizing) axis (table 2). However, the randomization test (Reynolds et al. 2010) showed that only the convex (negative eigenvalues) axes were significant in either sex (male γ_i : $P = .004$; female γ_i : $P = .014$), with the loadings on the eigenvectors having opposing signs for protein and carbohydrate (table 2). For males, protein loaded much more strongly than carbohydrate (near twofold) to the convex axis, whereas protein and carbohydrate loadings were of a similar magnitude for the convex axis in females (table 2). This suggests that

Table 1: Linear and nonlinear relationships between male and female fitness and macronutrient consumption in *Drosophila melanogaster*

	$\bar{X}(\sigma)$	$e(\beta)$	γ	
			Protein	Carbohydrate
Males (β_m, γ_m):				
Protein	107.54 (114.56)	5.36×10^{-4}	$-6.95 \times 10^{-5**}$	
Carbohydrate	210.29 (104.14)	$3.17 \times 10^{-3*}$	$5.7 \times 10^{-5**}$	-9.28×10^{-7}
Females (β_f, γ_f):				
Protein	155.55 (151.74)	-1.22×10^{-4}	-1.02×10^{-5}	
Carbohydrate	341.85 (157.67)	$2.46 \times 10^{-3***}$	$1.34 \times 10^{-5**}$	-1.02×10^{-5}
Sexes combined (β, γ):				
Protein	130.24 (135.49)	-6.6×10^{-4}	$3.0 \times 10^{-5*}$	
Carbohydrate	272.49 (147.54)	-7.1×10^{-4}	$-4.0 \times 10^{-5*}$	-4.63×10^{-6}

Note: Shown are the linear (β) and quadratic/correlational (γ) partial regression coefficients estimated in equation (2). The linear estimates were taken from a reduced version of equation (1), which included only the linear terms. Values for γ_{ii} have been doubled as required (Lande and Arnold 1983; Stinchcombe et al. 2008). Also shown are the partial regression coefficients from the sequential model building procedure comparing males and females. Shown are the interactions between the linear gradients and sex and between the quadratic and correlational gradients and sex. We have included the sex-specific population means (\bar{X}) and variances (σ), which can be used to transform linear (mean standardization: $\beta_\mu = \bar{X} \odot \beta$; variance standardization: $\beta_s = \sigma \odot \beta$) and nonlinear (mean standardization: $\gamma_\mu = \gamma_i \oslash (\bar{X}\bar{X}^T)$; variance standardization: $\gamma_s = \gamma_i \oslash (\sigma\sigma^T)$) estimates to different scales (Hansen and Houle 2008).

* $P < .05$.

** $P < .01$.

*** $P < .001$.

the sex difference detected by the model building procedure was due to a fundamental difference in the relative importance of protein and carbohydrate for males and females (fig. 1).

Dietary Preference

Likelihood ratio tests indicated significant genetic variance for dietary preferences in this population (G : LRT difference $-2\ln L = 70.8$, $df = 10$, $P \leq .001$). We further confirmed the presence of genetic (co)variance for dietary preference in each sex by running single-sex REML models (G_m : LRT difference $-2\ln L = 20.4$, $df = 3$, $P \leq .001$; G_f : LRT difference $-2\ln L = 49.8$, $df = 3$, $P \leq .001$).

Broad-sense heritabilities were higher in females than males for both macronutrients and were generally lower for protein than carbohydrate consumption (H^2 females: protein = 0.185, carbohydrates = 0.300; H^2 males: protein = 0.025, carbohydrates = 0.191). The genetic correlations between carbohydrate and protein consumption were positive and moderate, with similar values for correlations within and between the sexes (table 3). Most of the genetic variance for carbohydrate consumption was shared between males and females, resulting in a strong positive cross-sex genetic correlation that could not be distinguished from 1 (LRT; difference $-2\ln L = 0.2$, $df = 1$, $P = .655$). By contrast, protein consumption was only weakly correlated between males and females, and the genetic correlation could not

be distinguished from 0 (LRT; difference $-2\ln L = 0.4$, $df = 1$, $P = .527$).

The population mean preferences for the two macronutrients showed that when unrestricted, males (protein $\bar{X} = 6.44 \mu\text{g}$; carbohydrate $\bar{X} = 292.95 \mu\text{g}$) and females (protein $\bar{X} = 20.68 \mu\text{g}$; carbohydrate $\bar{X} = 413.50 \mu\text{g}$) consume much lower amounts of protein than carbohydrates, with consumption P : C ratios of around 1 : 20 in females and 1 : 45 in males; this suggests that both sexes target food sources rich in carbohydrates over protein. However, the ridge analysis indicated that both sexes could increase their competitive fitness by consuming more protein, feeding instead at a P : C ratio of around 1 : 2.1 in females and 1 : 2.5 in males (table 3). Despite both sexes being somewhat displaced from this optimal feeding ratio, there was a surprisingly large amount of genetic variance available in the population for an adaptive response. The unconditional evolvability ($e(\beta) = 2,684$) in the direction of optimal feeding response significantly exceeded the average evolvability ($\bar{e} = 1,395$) by a margin of 50% (mean difference between $e(\beta)$ and $\bar{e} = 1,290$; 95% HPDI: 618, 2,070) and was significantly lower than the average maximum evolvability ($e_{\max} = 5,130$) by a similar margin (mean difference between $e(\beta)$ and $e_{\max} = 2,445$; 95% HPDI: 1,355, 3,852).

Discussion

Nutrient acquisition and allocation are important determinants of variation in fitness components and phenotypic

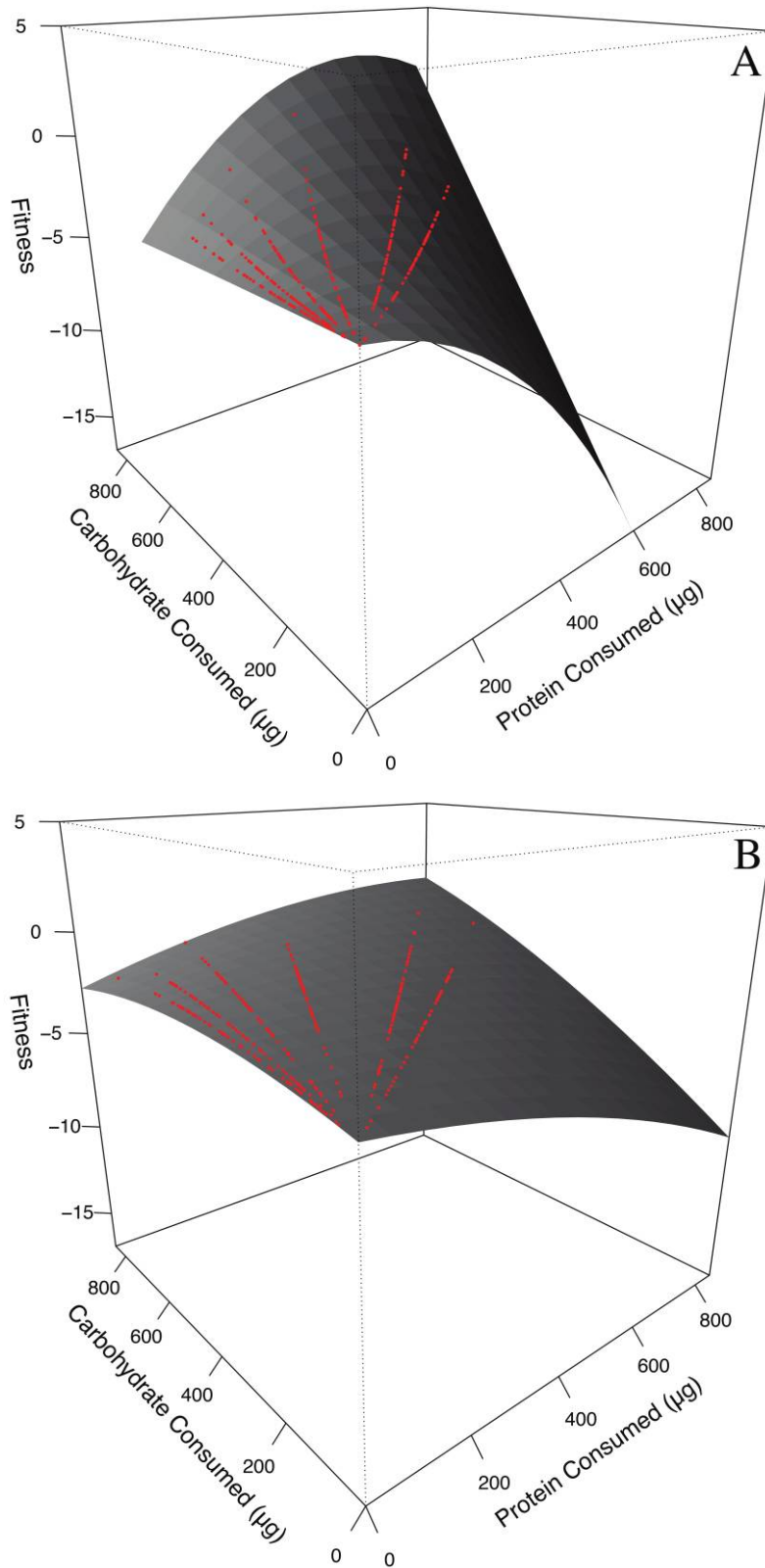


Figure 1: Three-dimensional representation of male (A) and female (B) fitness surfaces. Predicted fitness values for the observed consumption by individual flies are shown as red circles.

Table 2: Canonical analysis of the γ matrix for males and females

Eigenvalue	Permutation P	Eigenvectors	
		Protein	Carbohydrate
Males:			
3.13×10^{-5}	.055	.4922	.8704
-1.02×10^{-4}	.004	.8704	-.4922
Females:			
3.27×10^{-6}	.435	.7069	.7073
-2.37×10^{-5}	.014	.7072	-.7069

Note: Estimates for quadratic parameters of the consumption of protein and carbohydrate are from response surface models (table 1). P values were estimated using a randomization test (Reynolds et al. 2010) with 1,000 permutations.

trait expression (Lee et al. 2008; Maklakov et al. 2008; Fanson and Taylor 2011; South et al. 2011). However, despite evidence that physiological adaptations across generations can occur when organisms are exposed to experimentally altered diets (Warbrick-Smith et al. 2006), the potential for nutritional preferences to adapt genetically is not as well understood. By combining geometric and quantitative genetic frameworks, we have been able to examine both the effect of diet composition on fitness and the nature of standing variance for specific nutrient preferences. Using the theoretical framework put forward by Hansen and Houle (2008), we have shown that the evolutionary potential of preferences was well above average in this population. Furthermore, we have demonstrated that adult diet has important implications for the fitness of both male and female flies and that sex differences exist in the overall shape of the fitness surface. However, despite sex differences in dietary optima and the presence of strong cross-sex genetic correlations for dietary preference, there is currently little sexually antagonistic constraint in the expected direction of selection.

The acquisition and consumption of protein by adult females is a critical factor for egg production in most Diptera, including *Drosophila melanogaster* (Wheeler 1996; Lee et al. 2008). In this study, we found that the estimated female response surface conformed to this expectation, resembling surfaces from previous studies examining female reproductive fitness in other insects (Lee et al. 2008; Maklakov et al. 2008; Fanson and Taylor 2011). However, in contrast to male insect studies using geometric response surface estimation (Maklakov et al. 2008; South et al. 2011), our analyses indicated that fitness has a more complex nonlinear relationship with protein intake. Previous findings generally suggest that male insects require large amounts of carbohydrates and relatively little protein to maximize fitness, as a result of their need to compete with other males, display their suitability and attractiveness, and

overcome any female resistance in order to gain mating success (Long and Rice 2007; Maklakov et al. 2008; South et al. 2011). In contrast, our results suggest that males maximize their fitness at a P : C ratio nearly as great as the ratio that maximizes female fitness. Although the results in our study point toward protein intake having an important influence on male fitness, this does not necessarily conflict with these previous studies. One potential reason for the increased need for protein could be due to the synthesis of accessory gland proteins secreted by male *Drosophila* in their seminal fluid. The secretion of accessory gland proteins during mating has been shown to have striking effects on male paternity (Chapman 2001; Wolfner 2002), and the amount of protein consumed can affect the production of accessory gland proteins in *D. melanogaster* (Fricke et al. 2008). So while carbohydrates likely play a role in precopulatory sexual selection in males (Long and Rice 2007; Maklakov et al. 2008; South et al. 2011), protein may influence postcopulatory mating success (Fricke et al. 2008), both of which are incorporated in our competitive fitness assay.

Despite the population of lines (DGRP) used for the experiments in this article being maintained under standard laboratory dietary conditions, the genetic variation among these lines is representative of a natural population. The flies used to create the DGRP lines were sampled from a single outbred population in North Carolina (Mackay et al. 2012) and were subject to inbreeding soon after capture, which prevents large-scale adaptation to a laboratory environment. We found strong between-sex genetic covariance for preferences, suggesting that the same genes in males and females control variation in dietary preference traits. Yet despite also finding sex differences in the shape of the fitness surface, the direction of optimal response from our ridge analysis was similar between males and females (table 3). This suggests that although the potential for sexually antagonistic pleiotropy to constrain the independent evolution of the sexes exists, it may not have a major influence on evolutionary trajectories in this system because the ridge analysis suggests that both sexes are similarly displaced from their respective optima. The results presented in this article are similar to the findings of Maklakov et al. (2008), who showed phenotypic differences in the optima for males and females and a maladaptive response in dietary preference, suggesting that shared genetic variance between the sexes could be constraining their independent evolution. However, we have also shown that the direction of optimal response for feeding preferences is most closely aligned with g_{\max} (angle between vectors: $\theta_{g_{\max}} - \beta = 44.19^\circ$) than other axes of genetic variance ($\theta_{g_2} - \beta = 77.44^\circ$, $\theta_{g_3} - \beta = 60.38^\circ$; for the eigenanalysis of G , see table A3), reflecting the ap-

Table 3: Genetic variance-covariance matrix for male and female diet preference estimated using multivariate linear mixed-effects model fitted using the Mixed procedure in SAS (ver. 9.3)

	Male		Female		Vector of optimal response
	Protein	Carbohydrate	Protein	Carbohydrate	
Male:					
Protein	20.08	.59	.28	.48	183.36
Carbohydrate	112.57	1,788.27	.35	.95	181.14
Female:					
Protein	16.01	188.40	161.92	.40	301.49
Carbohydrate	122.23	2282.18	287.31	3,208.25	259.51

Note: Top left panel: G_m with variances in bold along the diagonal, covariance below the diagonal, and the correlation above the diagonal. Bottom right panel: G_f with variances in bold along the diagonal, covariance below the diagonal, and the correlation above the diagonal. Bottom left panel: between-sex covariance matrix (B). Top right panel: between-sex trait correlations; the correlations along the diagonal are equal to the intersexual genetic correlation, r_{mf} . Vector of optimal response calculated from the RIDGE analysis in SAS (ver. 9.3; see “Methods”).

parently similar displacement of both sexes from their optimal dietary preference in this population.

Our preference data from experiment 2 indicated that at a population level, both males and females focused on attaining carbohydrates, which suggests a strong preference for a low P : C ratio. While the mean female P : C consumption ratio was high at around 1 : 20, males were even more extreme at 1 : 45. These estimates appear quite different from the P : C ratio of approximately 1 : 2 required to maximize competitive fitness in males and females (fig. 1). The preference for the sucrose solution over the yeast solution in our results suggests that when protein and carbohydrate are available ad lib., there was a stronger preference to consume far larger amounts of carbohydrate compared with protein, despite the fitness benefits associated with a more balanced intake in both males and females. We also found evidence for this preference for carbohydrates over protein in our no-choice design; an individual fly’s desire for carbohydrates seemed to dictate the amount of food ingested (fig. A1). These two findings suggest that flies may only passively consume protein as they actively seek carbohydrate, despite the importance of both macronutrients to competitive fitness.

Although our preference trials showed that both males and females effectively overconsumed carbohydrates, neglecting protein, there are some caveats. First, we tested isolated virgin flies that may have been feeding to increase life span rather than aiming to improve reproductive fitness, which previous studies have shown to trade off against reproductive fitness (Lee et al. 2008; Maklakov et al. 2008; South et al. 2011). Second, DGRP flies are not lab adapted, so they may be expressing preference for carbohydrates over protein as a result of carbohydrates being the major limiting factor in their natural diet. It is likely that the feeding tubes in our experiment represent a richer source of nutrients than adult feeding sites in wild pop-

ulations. The role of yeast as the major source of nutrition in the diets of adult *Drosophila* has been well established (Sang 1978; Begon 1982), but yeast concentrations in wild-caught *Drosophila* are expected to be far lower than laboratory fed flies (Begon and Shorrocks 1978). As adults, *Drosophila* can survive (but not reproduce) with access to only simple sugar energy (Begon 1982), and reduced carbohydrate intake will lead to a decrease in life expectancy (Lee et al. 2008). The underlying nutritional value of any yeast is likely affected by the physical and chemical characteristics of the substrate (Hunter and Rose 1971; Sols et al. 1971; Begon 1982), with yeast odors—rather than the substrate—acting as the main attraction for *D. melanogaster* seeking out suitable breeding sites (Becher et al. 2012). Therefore, flies seeking feeding sites could prioritize consumption of yeasts that maximize carbohydrate intake, with protein passively consumed as a result. In either case, the genetic variance estimates suggest that this population should be able to adapt to environmental changes in nutrient availability, as shown by above average evolvability in the direction of highest fitness.

Our interpretation is based on the assumption that our competitive fitness assays are correlated with total fitness in the population from which the DGRP lines are derived. Our assays estimate the competitive performance of young flies over a period of 24 hours and therefore do not take into account the possible effects of variation in longevity and ageing, which can have important effects on net fitness. However, because we used non-lab-adapted flies, and because our primary aim was to gauge genetic constraints on adaptation and estimate evolvability in the natural source population, our performance assays are likely to provide more relevant estimates of fitness than longer-term measures of performance. In laboratory populations of insects, longevity is greatly extended relative to their counterparts in natural populations, which are subject to pre-

dation and parasitism, face shortages of food and water, and experience harsh weather (Kawasaki et al. 2008). Indeed, mean life expectancy in natural populations of some flies is only a few days (Bonduriansky and Brassil 2002; Kawasaki et al. 2008), whereas captive flies typically survive for 1–3 months (Finch 1994). Consequently, variation in performance that manifests late in life is likely to have much less impact on net fitness in natural populations than in the laboratory, and longitudinal or lifetime estimates of relative fitness under benign laboratory conditions may be unrepresentative of patterns in natural populations. Our early-life estimates of competitive performance are therefore likely to capture an important part of the variation in net fitness in the natural source population, allowing us to estimate the potential for genetic constraints and evolvability on dietary preference traits.

While individual animals have been shown to regulate intake from multiple food sources over their lifetime, compensating for changes in the availability of specific nutrients (Edgecomb et al. 1994; Raubenheimer and Jones 2006; Sørensen et al. 2008), the ability of a population to respond to intergenerational changes in nutrient availability has not been addressed. We have shown that flies have not only the phenotypic capacity to regulate their intake of both protein and carbohydrate separately, as has been found in other insect studies using the geometric framework (Raubenheimer and Simpson 1997; Lee et al. 2008; Maklakov et al. 2008; South et al. 2011), but also an underlying genetic basis to dietary preference that is largely shared by males and females. Preferences are expected to reflect the availability of different macronutrients within an environment, so large environmental fluctuations in resource availability are likely to change the structure of dietary preferences. Preferences in this population have an above average evolvability; thus, standing genetic variance is available to respond to shifts in macronutrient availability across generations. Our results suggest that it is likely that the underlying genetic traits for dietary preferences in *D. melanogaster* are not sex specific in expression, meaning that the potential for conflict will exist in situations when very different nutritional compositions maximize male and female fitness.

Acknowledgments

We thank S. Nuske for lab assistance and R. Brooks for discussion on the fitness surface analysis. T.P.G. was supported by a Marie Curie Inter-European Fellowship within the Seventh European Community Framework Program (PIOF-GA-2009-237271). S.F.C. and R.B. were supported by fellowships from the Australian Research Council. Fur-

ther funding for this research was also provided by the University of Queensland.

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Associate Editor: Anurag Agrawal
Editor: Mark A. McPeck



A feeding fruit fly. Photograph by A. O'Toole.