Genotype-by-sex-by-diet interactions for nutritional preference, dietary consumption and lipid deposition in a field cricket

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Running Title: G:Sex:Diet Interactions

Word Count Main Body: 6751

Abstract

The over-consumption of calories has led to a rise in the rates of obesity, diabetes and other associated disorders in both humans and a range of other species. While there is a genetic basis for regulating dietary intake and weight gain, genes rarely act in isolation and understanding the relative contribution of genes and the environment to food selection and lipid deposition remains a major challenge. By combining nutritional geometry with quantitative genetics, we determined the effect of genes, the nutritional environment and their interaction on the total nutritional preference (TP), total diet eaten (TE) and lipid mass (LM) of male and female black field crickets (*Teleogryllus commodus*) fed one of four diet pairs (DPs), that differed in their protein to carbohydrate ratio and total nutrition. We found abundant additive genetic variance for TP, TE and LM in both sexes and across all four DPs, with significant genetic correlations between TE and TP and between TP and LM in males. We also found significant genotype-by-DP and genotype-by-sex-by-DP interactions for each trait and significant genotype-by-sex interactions for TE and LM. Complex interactions between genes, sex and the nutritional environment, therefore, play an important role in nutrient regulation and lipid deposition in both males and females.

Keywords: Genotype-by-Environment Interactions, Genotype-by-Sex Interactions, Lipids, Nutrient Regulation, Obesity, *Teleogryllus commodus*

Introduction

The overconsumption of excessive calories has been associated with the rise in worldwide rates of obesity, cardiovascular disease, diabetes and other disorders and diseases in a range of animal species, including humans (Raubenheimer *et al.*, 2015). This overconsumption is puzzling because optimal foraging theory predicts that animals should evolve regulatory foraging mechanisms to optimize their evolutionary fitness (Stephens and Krebs, 1986; Simpson and Raubenheimer, 2012). Traditionally, theory has assumed that optimizing fitness required maximising energy intake (Stephens and Krebs, 1986). However, more recent developments using nutritional geometry have found that optimizing fitness requires animals to regulate both their energy intake and the specific balance (or ratio) of nutrients this energy comes from (Simpson and Raubenheimer, 2012).

Nutritional geometry is a multidimensional nutritional framework that entails varying the concentrations and ratios of nutrients in the diet and then accurately measuring their intake in feeding trials. This allows the construction of fine scale nutritional surfaces upon which a trait of interest can be mapped to determine the effect of and interaction between dietary components on the trait of interest (Simpson and Raubenheimer, 2012). A number of studies utilizing nutritional geometry have identified a number of different foraging mechanisms, for example compensatory feeding from a single diet or foraging from different nutritionally imbalanced foods, to maintain a constant and optimal intake of nutrients (Simpson and Raubenheimer, 2012). Examples of active nutrient regulation can be found in a number of species (e.g. predatory ground beetles (*Anchomenus dorsalis*) (Jensen *et al.*, 2012); fruit flies (*Drosophila melanogaster*) (Lee *et al.*, 2008; Jensen *et al.*, 2015); speckled roaches (*Nauphoeta cinerea*) (South *et al.*, 2011; Bunning *et al.*, 2015, 2016); and black field crickets (*Teleogryllus commodus*) (Maklakov *et al.*, 2008)), although, this regulated intake is not always optimal for the maximal expression of the traits examined in these studies (Maklakov *et al.*, 2008; Bunning *et al.*, 2015, 2016; Jensen *et al.*, 2015). However, sub-optimal nutrient regulation may just reflect an active compromise, whereby individuals regulate their intake of nutrients to balance the expression of multiple traits (Lihoreau *et al.*, 2015), which is perhaps not surprising given that different traits have been shown to have differing, trait-specific nutritional optima (e.g. Lee *et al*., 2008; Maklakov *et al*., 2008; Jensen *et al*., 2015). Alternatively sub-optimal regulation may indicate a constraint

in feeding behaviour through, for example, dietary assimilation, digestion, absorption and/or utilization (Henson and Hallam, 1995), with the efficiency of these processes linked to gut morphology (McWhorter and del Rio, 2000).

Despite this evidence for active nutrient regulation by animals, very little is known about the background genetic architecture that controls nutrient intake. There is support from studies on humans and rodent models that macronutrient intake has a genetic basis (Liu and Lloyd, 2013) and some evidence for genetic variation over food intake in *D. melanogaster* (Reddiex *et al.*, 2013; Garlapow *et al.*, 2015). Furthermore, a number of studies have identified candidate genes affecting variance over food intake with phenotypic variation over food intake. This variation was due to multiple segregating loci with alleles sensitive to environmental effects (Falconer and Mackay, 1996; Garlapow *et al.*, 2015, 2016), indicative of interactions between genes and the environment.

While we lack a complete understanding of the genetic background for nutrient intake, an important finding for the field of nutritional ecology is that animals, ranging from insects to mammals, have separate appetite systems for the intake of protein, carbohydrate and fat (Raubenheimer and Simpson, 1997; Gosby *et al.*, 2014). Specifically, when restricted to a diet of fixed macronutrient intake, animals regulate their intake of protein more strongly than carbohydrate and/or fat, through what has been termed the Protein Leverage Hypothesis (PLH) (Simpson and Raubenheimer, 2005; Sørensen *et al.*, 2008). The PLH postulates that when the proportion of protein contained in a diet is reduced, the powerful protein appetite stimulates an increased consumption of diet, in an attempt to gain more of the limited supply of protein. Accordingly, any dietary shift towards foods that are higher in carbohydrate and/or fat will dilute the availability of protein and increase the consumption and overall intake of energy (Simpson and Raubenheimer, 2005). It has been argued that the PLH can, therefore, explain the rise in levels of obesity and disease because of a shift (particularly in humans) towards consuming energy-dense foods that are high in carbohydrates and/or fats but low in protein (Brooks *et al.*, 2010; Gosby *et al.*, 2014; Raubenheimer *et al.*, 2015). The strength of the protein appetite is thus stimulating the increased intake of these energy dense foods and exposing individuals who carry obesity related genes (Shawky and Sadik, 2012; van der Klaauw and Farooqi, 2015) and are more susceptible to environmental changes, to the deleterious effects of excess caloric intake (O'Rahilly and Farooqi, 2006; Speakman, 2007; Corella and Ordovas, 2009; Reed *et al.*,

2010), with examples found in a number of taxa (e.g. spider monkey (*Ateles chamek*) (Felton *et al.*, 2009); humans (Gosby *et al.*, 2011, 2014; Martens *et al.*, 2013); mice (Sørensen *et al.*, 2008)).

There is however, variation in the susceptibility of individuals to these deleterious effects (van der Klaauw and Farooqi, 2015). Such variation would support the idea that how an individual regulates its dietary intake and the effect this has on fat deposition depends not only on the independent effects of genotype but the interactions between these genes and the nutritional environment. The differing response of a genotype in alternate (nutritional) environments, referred to as genotype-by-environment interactions (GxEs) are expected to be important, with their presence indicating that certain individuals are genetically pre-disposed to regulate their nutrient intake or deposit lipids in a specific way depending on variation in the nutritional environment. Some evidence of GxEs over dietary intake and obesity or its related disorders have been identified (Sutton *et al.*, 2006; Gordon *et al.*, 2008; Reed *et al.*, 2010) however, the diets used in these experiments are limited in that they lack the detail of specific nutrients and caloric intake to fully understand the interactions between active regulation in different dietary environment and the effects of this might have on obesity.

In addition to GxEs over dietary intake and fat deposition, one must also take into account the different nutritional requirements of males and females (Maklakov *et al.*, 2008; Simpson and Raubenheimer, 2012; Harrison *et al.*, 2014; Jensen *et al.*, 2015) resulting from different reproductive strategies. In most sexually reproducing species, males typically allocate more resources to mate competition while females typically allocate more resources to offspring production (Trivers, 1972). This results in males and females having different nutritional requirements, and drives the evolution of sex-specific nutritional optima for reproduction (e.g. *D.melanogaster* (Jensen *et al.*, 2015); *T.commodus* (Maklakov *et al.*, 2008)). Furthermore, sexual selection can promote sexual divergence in the strength and direction of nutritional trade-offs between various life-history traits, for example lifespan and reproduction (Lee *et al.*, 2008; Maklakov *et al.*, 2008; Reddiex *et al.*, 2013; Jensen *et al.*, 2015). How males and females actively regulate their intake of nutrients will determine the optimal expression of multiple fitness related traits (Simpson and Raubenheimer, 2012) and may also influence the interactions between genotype and the nutritional environment, resulting in significant genotype-by-sex-by-environment

149 interactions. It is important to note, however, that even if the sexes respond to the
150 nutritional environment in the same manner, males and females may still be genetically pre-
151 disposed to regulate their nutrient intake or deposit lipids in different ways if the underlying
152 physiological processes that regulate these traits are sex-specific, which will result in
153 significant genotype-by-sex interactions (North *et al*., 2007).

154 Here we combine nutritional geometry with quantitative genetics to determine how
155 male and female black field crickets (*Teleogryllus commodus*) of known genetic relatedness
156 respond when placed into four different nutritionally imbalanced environments. If
157 individuals are actively regulating their intake of nutrients, we predict that there will be
158 differences in the total amount of diet eaten and total nutrient preference across diet pairs
159 and this will influence lipid deposition. Moreover, if males and females differentially
160 regulate their intake of nutrients, we predict that the total amount of diet eaten and total
161 nutrient preference will differ across the sexes, as will the relationship between these traits
162 and lipid deposition. Finally, if nutrient regulation is under genetic control we predict that
163 there will be significant additive genetic (co)variance within and between these traits in
164 both sexes, as well as complex interactions between genotype, diet pair and sex (i.e.
165 genotype-by-diet pair, genotype-by-sex and genotype-by-sex-by-diet pair interactions).
166

167 ## Materials and Methods

168 *Study Species*

169 A total of 200 mated female *T. commodus* were collected from Smith's Lake, New
170 South Wales in eastern Australia in March 2009 and used to establish a large panmictic lab
171 population, maintained in 10 large culture containers (100L) of approximately 500 animals
172 per culture for 10 non-overlapping generations prior to this experiment. Lab populations are
173 kept at 28°C ± 1°C, under a 13:11 light:dark cycle, cleaned weekly and provided with
174 cardboard for shelter, water *ab libitum*, egg pads consisting of damp cotton wool and a
175 mixture of cat food (Purina Go Cat Senior©, St Louis, MO, USA) and rat food (SDS Diets,
176 Essex, UK). Nymphs were moved at random between culture containers each generation to
177 ensure gene flow.
178

179 *Artificial Diets*

Using the protocol established in South et al (South *et al.*, 2011) we made four powdered, holidic (i.e. chemically defined) diets. These four diets were used to make four dietary pairs, with each pair containing one diet with a P:C ratio of 1:8 and one with a P:C ratio of 5:1. We provided these diets in one of two nutritional dilutions (%P+C content), 36% or 84%. The four diet pairs (DPs) are as follows: DP1: 1:8 (36%) versus 5:1 (36%), DP2: 1:8 (84%) versus 5:1 (36%), DP3: 1:8 (36%) versus 5:1 (84%), and DP4: 1:8 (84%) versus 5:1 (84%) with composition also provided in Table S1. These diets were selected from a larger geometric array of a possible 24 diets because they provide a broad coverage of potential nutrient space (Figure S1) and have been used in previous choice feeding experiments (South *et al.*, 2011; Bunning *et al.*, 2015).

Quantitative Genetic Breeding Design

To estimate the quantitative genetics of total diet eaten, nutritional preference and lipid mass, we used a split-brood half-sib breeding design whereby sons and daughters from each full-sib family were split across four different diet pairs and their intake of nutrients measured under dietary choice for 21 days. The half-sib breeding design was established by mating each of 30 randomly chosen virgin sires with three randomly chosen dams. A total of 50 offspring from each dam were collected and reared in a family group in an individual plastic container (10 x 10 x 5cm) for three weeks, with access to an *ad libitum* supply of ground cat food (Purina Go Cat Senior©, St Louis, MO, USA) and water provided in a 5cm plastic tube plugged with cotton wool. After three weeks, 12 sons and 12 daughters per dam were isolated and established at random in individual plastic containers (5cm x 5cm x 5cm) and provided with *ad libitum* cat food pellets and water, and checked daily for eclosion to adulthood. Containers were cleaned and fresh food and water were provided weekly. On the day of eclosion, we randomly allocated 3 sons and 3 daughters per dam to each of four diet pairs (total n = 1080 sons and 1080 daughters; see Fig S2 for a graphical representation of our breeding design). Fresh diet was provided every three days for a total of 21 days (i.e. a total of seven feeding periods). Experimental animals were mated with a stock animal of the opposite sex on the evening of day 8 post-eclosion and removed on day nine with females provided with a petri dish of moist sand thereafter for oviposition.

Feeding Regime

Experimental feeding followed established protocols used previously (South *et al.*, 2011). In brief, two dishes of diet of measured dry weight were provided to each cricket according to assigned diet pair. Food was provided in feeding platforms constructed by gluing the upturned lid of a vial (1.6 cm diameter, 1.6cm deep) onto the middle of a petri dish (5.5 cm diameter) and water was provided *ad libitum* in a 5ml test tube plugged with cotton wool. Any diet spilled during feeding was collected in the petri dish and weighed. All diets were dried in an oven (Binder FD115, Germany) at 30°C for 72 hrs before weighing. Feeding platforms were weighed before and after each feeding period using an electronic balance (Ohaus Explorer Professional EP214C, Switzerland). Faeces were removed from the diet and feeding platform using forceps prior to re-weighing. Diet consumption was calculated as the difference in dry weight of diet before and after feeding. This amount of consumed diet was converted to a weight of P and C ingested by multiplying by the proportion of these nutrients in the diet (South *et al.*, 2011).

Measuring Lipid Mass

On day 21, crickets were frozen at -20°C and stored until total body lipid analysis could be performed. Lipid extraction was performed using the protocol outlined in South *et al.* (2011). In brief, each cricket was defrosted to room temperature and a slit was made along the abdomen using dissecting scissors. The cricket was then dried at 60°C for 24 hours and weighed using an electronic balance. Each cricket was then placed in 10ml of a 2:1 (v/v) solution of dichloromethane:methanol and agitated for 48 hrs to extract lipids. Crickets were then removed from this solution and dried for a further 24 hours at 60°C and then weighed. The difference between the pre- and post-extraction weights of each cricket was taken as the lipid mass.

Statistical Analysis

Quantitative genetic analyses were performed using animal models fitted in ASReml (version 3) (Gilmour *et al.*, 2009). An animal model is a form of linear mixed-effect model incorporating pedigree information where an individual's genetic merit is included as a random effect allowing for the estimation of the additive genetic (co)variance (Wilson *et al.*, 2010). We examined three phenotypic traits: the total amount of diet eaten (TE) (including nutritional and non-nutritional components), total nutritional preference (TP) (calculated as

total protein intake divided by total carbohydrate intake) and total body lipid mass (LM) (as a measure of fat deposition). Prior to analysis each trait was standardized to a mean of zero and standard error of one using a *Z*-transformation and body size (measured as the width of the pronotum) was included as a fixed effect in all models to control for any size effects on TE, TP or LM.

We first tested for the effect of sex and DP on our three traits using Wald-*F* tests. Given the significant effect of sex and DP on all three traits (see Results) we included these as fixed effects in a univariate model and estimated the additive genetic variance (V_A) for each trait by comparing univariate models run without and with the addition of the breeding values as a random effect for each trait. We then examined the presence and strength of any interactions between G and the dietary environment and between G and sex. We tested for a G-by-DP interaction by running univariate models for each trait but split across the four DPs with sex included as a fixed effect. We similarly tested for a G-by-Sex interaction by running univariate models for each trait but split across the sexes with DP included as a fixed effect. In both cases, a secondary analysis was performed to explore sex and DP differences by restricting G-by-DP models to one sex at a time and restricting G-by-Sex models to one DP at a time. Finally, we tested for G-by-Sex-by-DP interactions by running univariate models for each trait split across each DP for males and females. We also extracted estimates of additive genetic (co)variances, heritabilities (h^2) and genetic correlations (r_A) from these models (Table 3), this represents a matrix (G) of the additive genetic variances (along-diagonal), covariances (below-diagonal) and correlations (above-diagonal). Model summaries and Log-likelihoods for all our quantitative genetic models can be found in Tables S2 and S3 and example ASReml code can be found in Text S1. Statistical inference was based on likelihood-ratio tests (LRT). Due to the greater mathematical complexity in fitting multivariate models with an increasing number of response variables, we were unable to run a single multivariate (multi-trait) model which included each trait split by sex and DP treatments (e.g. 3 Traits x 2 Sexes x 4 DPs = 24 Trait x Sex x DP combinations).

Finally, given the difference in TE, TP and LM across DPs (see Results) and the sexes we also explored the effects of P and C intake on LM and whether this differed across the sexes. We used a response surface approach to characterize the linear and non-linear (quadratic and correlational) effects of nutrients on LM in each sex (South *et al.*, 2011). We

visualised the effects of P and C in LM in each sex using thin-plate splines constructed using the *Tps* function in the FIELDS package of R (version 2.15.1, www.r-project.org). We then statistically compared the linear, quadratic and correlational effects of nutrient intake across the sexes using a sequential model building approach outlined in South *et al*. (2011).

Results

There was a significant effect of DP and Sex on TE, TP and LM (Table 1). For both sexes, TE was highest on DP1, followed by DP3, DP2 and lowest on DP4 which is consistent with compensatory feeding in the sexes. Males and females increased their consumption of diet by 58% and 72% respectively, when feeding on the lowest (DP1, 36% nutrition) versus the highest (DP4, 84% nutrition) nutrient DP. Females consumed more diet than males on each DP and their consumption of diets was, on average, 20% higher than males across all DPs. (Fig. S3.)

For TP, values for both sexes were highest for DP3, followed by DP1, DP4 and DP2 with TP values being greater for females than males on each DP. This can be visualized in Fig. 1, which shows the mean P and C intake of the sexes on each DP, as well as the regulated intake point (RIP) for each sex (calculated as the mean intake of these nutrients across DPs and represents the point in nutrient space that individuals actively defend when given dietary choice). With the exception of DP3, crickets on all other DPs showed a preference to consume relatively more C than P (Fig. 1), however this C biased preference was more prominent in males with a RIP at a P:C ratio of 1:2.02 than females with a RIP at a P:C ratio of 1:1.71 (Fig. 1), with non-random feeding, confirming active nutrient regulation, found for both sexes in all four DPs (Fig S4).

For both sexes LM was highest on DP4, followed by DP2, DP3 and DP1 (Fig. 2). Despite the higher consumption of diets by females, LM was actually higher in males than females (Fig. 2). Response surface analysis showed that LM increased linearly with the intake of C in both sexes and decreased linearly with P intake in males but not in females (Table 2). There were significant positive quadratic effects of P intake on LM in both sexes but no significant quadratic effects of C intake (Table 2). There was a significant negative correlational effect of nutrient intake on LM in males but not females (Table 2). The effect of nutrient intake on LM in the sexes is presented as thin-plate splines in Fig. 2 and they

confirm that LM is maximised at a high intake of C and low intake of P in both sexes. Indeed, a sequential model-building approach revealed that linear ($F_{2,2068}$ = 1.16, P = 0.31), quadratic ($F_{2,2064}$ = 2.33, P = 0.10) and correlational ($F_{1,2062}$ = 2.80, P = 0.10) effects of P and C intake on LM did not differ significantly between the sexes.

LRT tests found significant additive genetic variance for TE, TP and LM in each sex and across the four DPs (Models A-B, Table S2).We also found evidence for significant G-by-DP interactions for each trait with a univariate model containing just G significantly improved with the addition of a G-by-DP interaction term (Models C-D, Table S3). Further exploration within each sex shown that this interaction was significant for all three traits in both males and females, being especially pronounced for TP (Table S4). These interactions are visualized in the reaction norms provided in Fig. 3, with multiple crossovers signalling that different genotypes respond differently across DP, indicative of significant G-by-DP interactions. We also found evidence for significant G-by-Sex for TE and LM but not TP with univariate models for TE and LM significantly improved by the addition of a G-by-Sex interaction term (Models E-F, Table S3). Further exploration within each DP showed that this interaction was significant in all four DPs for TE and LM but was only significant in DPs 1, 2 and 3 for TP (Table S4). These interactions are visualized in the reaction norms provided in Fig. 4 with multiple crossovers signalling significant G-by-Sex interactions for each trait but more so for TE and LM than TP, especially in DP4.Finally, we found evidence for significant G-by-Sex-by-DP interaction for TE, TP and LM with the fit of univariate models was significantly improved by the addition of this interaction term (Models G-H, Table S3). This finding suggests that complex interactions between genes, sex and the nutritional environment are key to the intake of nutrients and lipid deposition in *T.commodus.* More specifically, it indicates that individuals are genetically pre-disposed to regulate their nutrient intake or deposit lipid but this depends on variation in the nutritional environment and their sex. A significant G-by-Sex-by-DP interaction also suggests that the additive genetic variance-covariance structure among these traits is also likely to change significantly with sex and DP. We provide estimates of the additive genetic variance in and covariance between these traits for each sex in the four DPs. With only the exception of TP for females in DP4, all h^2 estimates for the sexes in each DP were significantly greater than zero. There was, however, substantial variability in h^2 estimates, ranging from 0.25 to 0.94, and there was no clear pattern with regards to DP or sex. In contrast, estimates of genetic correlations

(r_A) between traits showed a number of clear differences across the sexes and DPs. First, estimates of r_A were more pronounced in males than females, with 9 estimates being significantly greater than zero in males, compared to only two in females (Table 3). Second, there is a significant positive r_A between TE and TP for all DPs in males, whereas this genetic correlation is only significant for DP2 in females (Table 3). Third, there is a significant negative r_A between TE and LM for DP1 in males but a significant positive r_A between these traits in DP3 (Table 3). In contrast, there is no significant covariance between TE and LM in females (Table 3). Finally, there is a significant negative r_A between TP and LM for DP1, DP2 and DP4 in males, but a negative r_A between these traits is only significant for DP1 in females (Table 3).

Discussion

In this study, we combined quantitative genetics and nutritional geometry to examine the interactions between genes and the dietary environment when male and female *T.commodus* encounter different nutritionally imbalanced environments and the consequences of these interactions on feeding behaviour, nutrient regulation and lipid deposition. We predicted that if *T. commodus* actively regulate their feeding behaviour and nutrient intake, there would be differences in TE and TP across DPs and this would have important implications for LM. Moreover, due to the divergence in the nutritional requirements of the sexes, we further predicted that any differences in TE and TP across DPs would be sex-specific, as would the relationship between TE, TP and LM. In agreement with these predictions, we found that male and female *T. commodus* showed considerable differences in TE and TP across DPs, consistent with active nutrient regulation. There were, however, clear sex differences with females consuming more diet and showing a stronger preference for the intake of P relative to C than males on each DP. Interestingly, despite their higher dietary consumption, females exhibited lower LM on each DP compared to males. Given their higher dietary consumption compared to males we would have expected a corresponding higher measure of LM for females since increased consumption has been shown to result in increased lipid deposition (Qi and Cho, 2008; Raubenheimer *et al*., 2015). We further predicted that if nutrient regulation is under genetic control, there will be significant additive genetic (co)variance both within and between these traits in both sexes,

as well as complex interactions between genotype, DP and sex. Consistent with this prediction, we show that there is ample additive genetic variance in TE, TP and LM in both sexes and across all DPs (the only exception being for TP in females in DP4), as well as substantial additive genetic covariance between these traits. This covariance between traits was more pronounced in males than females, most notable being the consistent positive genetic correlation between TE and TP, suggesting that genotypes associated with consuming more diet are also predisposed to having a preference for P, as well as the negative genetic correlation between TP and LM across DPs, suggesting that genotypes (G) associated with a preference for C, are predisposed to having higher LM. Most importantly, we provide evidence for significant G-by-DP and G-by-Sex-by-DP interactions for each trait, as well as significant G-by-Sex interactions for TE and LM but not TP. Together, our findings demonstrate that complex interactions between genotype, sex and the nutritional environment play a central role in how *T. commodus* regulate their feeding behaviour and nutrient intake in response to a nutritionally imbalanced environment with important implications for lipid deposition in the sexes.

Optimal foraging theory (Stephens and Krebs, 1986) predicts that when in a nutritionally imbalanced environment, an animal may actively regulate their intake of nutrients either through compensatory feeding or by eating non-randomly from multiple food sources (Simpson and Raubenheimer, 2012). Our finding that there is considerable variation in both TE and TP across DPs and the sexes suggests that both processes are operating in male and female *T. commodus* but to differing degrees. We found that both sexes increased the total amount of diet they consumed on the lowest nutrition pair (DP1, 36% nutrition) compared to highest nutrition pair (DP4, 85%) but this increase was larger in females (72%) than males (52%). While compensatory feeding has been demonstrated in a variety of animal taxa (Simpson & Raubenheimer 2012), only a few studies have reported sex differences in this behaviour and existing studies show that the magnitude of compensatory feeding is higher in males than in females (Barreto *et al.*, 2003). We also show that females have consistently higher TP values than males on each DP and although both sexes show an overall preference for C intake over P intake, the RIP was relatively more P biased in females (P:C ratio = 1:1.71) than males (P:C ratio = 1:2.02). This contrasts with earlier work in *T.commodus* that showed no sex-differences in the regulated intake of P and C (Maklakov *et al.*, 2008). The differences we observe in *T. commodus*, however, can be

explained by the divergent reproductive strategies of the sexes. Male *T. commodus* produce a metabolically demanding (Kavanagh, 1987) advertisement call that is used to attract females, with the amount of time spent calling being a major determinant of male mating success (Bentsen *et al.*, 2006). To fuel this signalling behaviour, males require a high intake of C which provides an abundant source of energy that is available rapidly after digestion and calling effort has subsequently been shown to be maximised at a P:C ratio of 1:8 (Maklakov *et al.*, 2008). Reproductive success in females, however, is largely determined by the number of eggs produced and P intake is known to play an important role in stimulating oogenesis and regulating vitellogenesis in insects (Wheeler, 1996). Females, therefore, require a higher intake of P relative to males to maximise egg production and the RIP of female *T. commodus* has been shown to be more P biased than in males (P:C = 1:1; Maklakov *et al*., 2008). It is important to note, however, that despite this sexual divergence, neither sex has been found to optimally regulate their relative intake of P and C to maximise reproductive success, although females do appear to regulate closer to the optimal P:C ratio than males (Rapkin *et al.*, 2017).

Current theories on the link between diet and obesity have highlighted the over ingestion of energy dense foods as a primary factor in weight gain (Mathes *et al.*, 2011; Raubenheimer *et al.*, 2015). While we cannot show 'over-ingestion' in our study, we do show that lipid deposition in male and female *T. commodus* was significantly greater on the DP with the highest total nutrition (DP4, 84% nutrition) and lowest on the DP containing lowest total nutrition (DP1, 36% nutrition). However, we also show that lipid deposition is not only contingent on the energy (caloric) content of the diet but also the relative intake of nutrients. This is illustrated by the difference in lipid deposition of both sexes when feeding from DP2 and DP3; both DPs contain the same total energy content, but the highest nutrient diet in DP2 is C biased (P:C = 1:8, 84% total nutrition) whereas it is P biased on DP3 (P:C = 5:1, 84% total nutrition). Consequently, the significantly higher lipid deposition of males and females feeding from DP2 than DP3 suggests that the intake of C is more important to lipid deposition than P intake. Our response surface analysis also shows that LM was maximised in both sexes at a high intake of C and low intake of P (Table 2, Fig. 2). This finding supports the well-established link between increased C intake and lipid deposition reported in a range of animal taxa (Mathes *et al.*, 2011; Raubenheimer *et al.*, 2015). It also explains the lower LM of females than males on each of the DPs, despite their higher overall

consumption of diets: by consuming relatively more P to C than males, female deposit lower levels of lipids. However, we cannot rule out other mechanisms that may explain this reduced LM in females, for example, egg production causes a substantial mobilization of lipid reserves from the fat body to the ovaries in insects (Ziegler and Van Antwerpen, 2006). It is, therefore, possible that females are utilizing more of their lipid stores to provision eggs, whereas males are using relatively less C for calling and storing the remainder as lipids. Unfortunately, our measure of LM measured the total lipids present in the entire body so we are unable to state how lipids were mobilized to specific organs/tissues such as eggs or specific lipid classes (e.g. triglycerides). Future studies would benefit from a more specific measure of lipid deposition as has been highlighted in studies looking at the production and deposition of lipids into somatic and reproductive organs in female flight vs flightless cricket morphs in *Gryllus firmus* (Zera, 2005). Alternatively, a simple solution at present would be to measure the LM of virgin females, with reduced egg production, on each of the DPs, to test this hypothesis (Nestel *et al.*, 2005).

The physiological systems that control lipid deposition rely on a highly complex, polygenic contribution of genes. There exist examples from a number of classic molecular genetic studies using mice (Marie *et al.*, 2000) and human models (Raubenheimer *et al.*, 2015) but there is also growing evidence using more recent genomic approaches in humans (e.g. Robbins and Savage, 2015) and *C.elegans* (e.g. Zhang *et al*., 2010). Further studies have also specifically looked at the genetic components of lipid acquisition, storage and mobilisation in five insect species (*D.melanogaster*, mosquitoes (*Anopheles gambiae*), honey bees (*Apis mellifera*), moths (*Bombyx mori*), and beetles (*Tribolium castaneum*) (Horne *et al.*, 2009), and between dimorphic wing morphs in the cricket *G.firmus* (e.g. Schilder *et al*., 2011; Nanoth Vellichirammal *et al*., 2014). The complexity surrounding lipid deposition is perhaps not surprising given that lipid deposition (and by extension obesity) is influenced by interactions between many variables, for example; environment (dietary and social) (Qi and Cho, 2008; Mathes *et al.*, 2011), microbiota (Schilder and Marden, 2006; Wolf and Lorenz, 2012), various life-history traits including reproduction and ageing (Hansen *et al.*, 2013) and other genes either related to feeding behaviour and lipid deposition (e.g. “thrifty gene hypothesis”) (Neel, 1962; Barsh *et al.*, 2000) and/or genetic pathways linked to other life-history traits (e.g. Insulin-like growth factor-1 (IGF-1) (Post and Tatar, 2016); mechanistic

target of rapamycin (mTOR) (Kapahi *et al.*, 2010) and nuclear hormone receptor-80 pathways (NHR-80) (Goudeau *et al.*, 2011)).

Our results are in broad agreement with the general view that lipid deposition is a complex trait that is influenced by the interaction between many variables. We show that LM in *T. commodus* is influenced by a complex interaction between genotype, the nutritional environment and sex. Furthermore, there is considerable additive genetic covariance between LM, TE and TP with the latter two feeding behaviours also subject to complex G-by-DP-by-Sex interactions. These findings demonstrate that to understand lipid deposition in *T. commodus,* it is not simply enough to characterize the independent contributions of the genotype, nutritional environment and sex to this trait: context is important. That is, these complex interactions in *T. commodus* mean that whether an individual is predisposed to increased lipid deposition cannot be predicted with complete accuracy from any one of these variables in isolation. Consequently, before any specific measures for obesity prevention that are tailored to an individuals' personalized genetic make-up will be effective (Qi and Cho, 2008), a better understanding of how these complex interactions regulate LM is essential.

Our results show an abundance of additive genetic variance for TE, TP and LM, in addition to a number of genetic correlations between these traits. This might suggest that the control of an individual's dietary and nutrient intake and how and individual stores dietary lipids might be genetically linked and possibly unable to evolve independently (Lande, 1980). However, further investigation using linkage-mapping or genome wide association studies would be required to determine the specific genes controlling these traits and how these genes might be linked. Our results do however, show a number of consistent patterns at the level of the genotype. Firstly, the number of significant genetic correlations between TE, TP and LM was greater in males than females (9 versus 2, respectively). h^2 estimates were large for all traits and there were no systematic differences in these estimates across the sexes indicates that this pattern is not due to a simple lack of additive genetic variance for these traits in females (with the notable exception of TP in DP4). This does suggest that either the genetic pathway regulating feeding behaviour and LM is different in the sexes or it is the same but more tightly regulated in males than females, although further investigation at the gene level would be needed to confirm this.

Secondly, there were consistent positive genetic correlations between TE and TP across all DPs in males and also in DP2 for females. In our study, TP was measured as the total intake of P divided by the total intake of C. Higher values of TP, therefore, mean a preference for more P relative to C, even when there is an absolute preference for C (TP < 1.0, and shown in DP1, 2 and 3 of Fig S2). Consequently, this positive genetic correlation indicates that in males and in some nutritional environments for females, the genes that govern the preference for P relative to C, are positively associated with the genes for dietary consumption. Finally, there were negative genetic correlations between TP and LM on DP1, 3 and 4 in males and DP1 in females. This indicates that the genes for LM are negatively associated with those governing the preference for P relative to C. Collectively, both of these patterns of additive genetic covariance between traits provide partial support for the PLH at the genetic level. The PLH predicts that in a nutritionally imbalanced environment where P is limited, the powerful P appetite will stimulate individuals to increase their dietary consumption in an attempt to gain more P (Simpson and Raubenheimer, 2005; Sørensen *et al.*, 2008; Gosby *et al.*, 2014), a pattern that is supported by the positive genetic correlation between TP and TE, where a preference to consume P is causing an increase in the TE, for example males in DP2 have a genetic correlation of 0.93 (±0.16) between TE and TP. DP2 is highly carbohydrate biased and so males seeking to increasing their P intake are consuming increasing amounts of the available diets. Furthermore, the PLH predicts that a side effect of attempting to consume a limited supply of P is the over-ingestion of more abundant nutrients (such as C) that cause increased lipid deposition and predispose an individual to obesity (Simpson and Raubenheimer, 2005; Sørensen *et al.*, 2008; Gosby *et al.*, 2014). The observed negative genetic correlations between TP and LM agree with this prediction, although it also supports the alternate view that the genes for C preference are directly linked to those for LM. Further support for this prediction would have come from positive genetic correlations between TE and LM, however, this relationship was inconsistent in males being negative in DP1 and positive in DP3.

In conclusion, while our work is in general agreement with the commonly held view that the consumption of energy rich diets is a major contributor to the increased rates of obesity in most developed societies, it also clearly demonstrates that the causes of increased lipid deposition are far more complex than this in *T. commodus*. Complex interactions between genotype, the nutritional environment and sex for feeding behaviour

(TE and TP) and LM, as well as additive genetic covariance between these traits, means that focussing on any one of these variables in isolation will provide an incomplete understanding on whether an individual is predisposed to lipid deposition (or obesity) or not. The obvious question that remains from our work is what are the consequences of high lipid deposition in male and female *T. commodus*? In humans, as well as a range of mammalian models, there is clear evidence that excessive lipid deposition and obesity are responsible for a number of different metabolic and cardiovascular disorders (Raubenheimer *et al.*, 2015) which negatively impact health. There is also growing evidence of similar disorders in insects (e.g. *Drosophila* (Musselman *et al.*, 2011) and dragonflies (*Libeullula pulchella*) (Schilder and Marden, 2006) which supports the suitability of using insects in obesity studies. There is also growing evidence in insects of the fitness costs of obesity (e.g. *Drosophila* (Skorupa *et al.*, 2008; Musselman *et al.*, 2011; Na *et al.*, 2013); *L.pulchella* (Schilder and Marden, 2006) and diamond back moth (*Plutella xylostella*) (Warbrick-Smith *et al.*, 2006), therefore, understanding the interactions between genetic mechanisms controlling feeding behaviour and lipid deposition, the environment and the resultant consequences on evolutionary fitness and long term health would clearly be a useful avenue for future obesity research.

Acknowledgements

JH was funded by a University Royal Society Fellowship and Equipment Grant and by NERC (NE/G00949X/1), AJW by a BBSRC Fellowship. JR was funded by a NERC studentship (NERC/1200242) awarded to JH.

Data Accessibility. All data will be deposited at dryad (www.datadryad.org) upon acceptance of this manuscript.

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719

Tables

Table 1. *F*-tests examining the significance of body size, sex and diet pair on our three trait measures: total eaten, total preference and lipid mass.

	F	*df*	P
Total Eaten			
Sex	407.92	1,2154	0.001
Diet Pair	527.45	3,2154	0.001
Total Preference			
Sex	2035.36	1,2154	0.001
Diet Pair	437.44	3,2154	0.001
Lipid Mass			
Sex	272.48	1,2154	0.001
Diet Pair	238.75	3,2154	0.001

Table 2. Response surface analysis quantifying the linear and nonlinear effects of protein (P) and carbohydrate (C) intake on lipid deposition in male and female *Teleogryllus commodus*. Significant (P<0.05) linear and nonlinear effects are highlighted in bold.

	Linear effects			**Nonlinear effects**		
Sex	P	C		P x P	C x C	P x C
Males						
Gradient ± SE	**-0.08 ± 0.03**	**0.52 ± 0.03**		**0.09 ± 0.02**	-0.00 ± 0.02	**-0.10 ± 0.03**
t_{1029}	**3.14**	**19.38**		**4.23**	0.06	**2.87**
P	**0.002**	**0.0001**		**0.0001**	0.95	**0.004**
Females						
Gradient ± SE	-0.03 ± 0.03	**0.49 ± 0.03**		**0.04 ± 0.02**	0.02 ± 0.03	-0.00 ± 0.03
t_{1041}	1.09	**18.12**		**2.00**	0.81	0.07
P	0.27	**0.0001**		**0.04**	0.42	0.95

Table 3. Additive genetic variance-covariance matrices (**G**) for total diet eaten (TE), total nutrient preference (TP) and lipid mass (LM) in males and females across the four diet pairs tested. Genetic correlations (r_A, in italics) are above the diagonal, additive genetic variances are along the diagonal and the additive genetic covariance between the traits are provided below the diagonal, with SEs for these parameters being provided in brackets. Heritability (h^2) estimates for each trait are provided in a separate column (with SEs provided in brackets). Significant estimates of r_A and h^2 are in bold where *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

	Males					Females			
	TE	**TP**	**LM**	**h^2**		**TE**	**TP**	**LM**	**h^2**
Diet Pair 1									
TE	0.38 (3.64)	***0.79 (0.09)******	***-0.56 (0.23)*****	**0.56 (0.13)****	**TE**	0.16 (1.91)	*-0.24 (0.26)*	*0.06 (0.29)*	**0.25 (0.12)***
TP	0.25 (4.19)	0.25 (5.28)	***-0.64 (0.15)******	**0.94 (0.12)*****	**TP**	-0.41 (-1.06)	0.18 (5.09)	***-0.54 (0.15)******	**0.80 (0.12)*****
LM	-0.15 (-2.49)	-0.13 (-3.33)	0.18 (2.71)	**0.57 (0.14)*****	**LM**	0.08 (0.22)	-0.74 (-3.01)	0.10 (3.27)	**0.49 (0.13)*****
Diet Pair 2									
TE	0.27 (3.92)	***0.93 (0.16)******	*-0.23 (0.23)*	**0.67 (0.13)*****	**TE**	0.36 (4.30)	***0.47 (0.15)******	*0.06 (0.22)*	**0.81 (0.13)*****
TP	0.49 (3.45)	0.01 (2.26)	***-0.55 (0.24)****	**0.31 (0.13)***	**TP**	0.48 (2.43)	0.29 (3.56)	*0.11 (0.25)*	**0.69 (0.15)*****
LM	-0.86 (-1.07)	-0.40 (-1.79)	0.51 (2.67)	**0.59 (0.14)*****	**LM**	0.15 (0.24)	0.08 (0.46)	0.19 (2.28)	**0.68 (0.13)*****
Diet Pair 3									
TE	0.30 (3.45)	***0.68 (0.18)******	***0.34 (0.20)****	**0.53 (0.13)*****	**TE**	0.31 (3.51)	*-0.02 (0.17)*	*0.09 (0.23)*	**0.65 (0.14)*****
TP	0.25 (3.25)	0.45 (3.67)	*-0.01 (0.20)*	**0.60 (0.13)*****	**TP**	-0.07 (-0.09)	0.66 (4.77)	*-0.21 (0.19)*	**0.93 (0.13)*****
LM	0.11 (1.61)	-0.02 (-0.03)	0.38 (3.35)	**0.39 (0.14)****	**LM**	0.16 (0.40)	-0.56 (-1.11)	0.11 (2.83)	**0.56 (0.13)*****
Diet Pair 4									
TE	0.24 (4.07)	***0.47 (0.22)****	*-0.31 (0.22)*	**0.72 (0.13)*****	**TE**	0.26 (4.12)	*0.84 (0.91)*	*0.00 (0.27)*	**0.77 (0.13)*****
TP	0.53 (1.90)	0.52 (2.15)	***-0.57 (0.26)****	**0.27 (0.12)***	**TP**	0.40 (1.72)	0.09 (0.48)	*-0.52 (0.85)*	0.06 (0.12)
LM	-0.11 (-1.44)	-0.97 (-1.83)	0.56 (2.66)	**0.59 (0.14)*****	**LM**	-0.00 (-0.00)	-0.17 (-0.65)	0.12 (1.61)	**0.79 (0.12)*****

Figure Legends

Fig. 1. The mean (±SE) intake of P and C by male (blue symbols) and female (red symbols) *T. commodus*. The open symbols represent the mean intake of nutrients in each of the four diet pairs (denoted by pair number), whereas the solid symbols represent the regulated intake point (RIP), calculated as the mean of the four diet pairs. The solid blue and red lines represent the nutritional rails (lines in nutrient space that represents a fixed intake of nutrients) that passes through the RIP for males (P:C ratio of 1:2.02) and females (P:C ratio of 1:1.71). The black dashed lines (P:C ratios of 5:1 and 1:8) represent the outer nutritional rails of the nutritional landscape.

Fig. 2. Thin-plate spline (contour view) visualizations of the effects of protein (P) and carbohydrate (C) intake on lipid mass in (A) female and (B) male *Teleogryllus commodus*. In each spline, the red regions represent higher values for the measured trait, whereas blue regions represent lower values. The white crosses represent the RIPs from Fig. 1 overlaid on the respective female and male landscapes. The black symbols represent the mean P and C intake of each sire within the four diet pairs.

Fig. 3. Reactions norms illustrating the genotype-by-diet pair interaction (G:DP) for the total amount of diet eaten (TE), total nutrient preference (TP) and lipid mass (LM) in male and female *T. commodus*. Females are presented with a grey background and males with a white background. Each column of the figure presents a specific diet pair comparison between the sexes for each trait. In each panel, lines represent the response of a given genotype across two diet pairs.

Fig. 4. Reaction norms illustrating the genotype-by-sex interaction (G:S) for the total eaten (TE), the total nutritional preference (TP) and lipid mass (LM) in the different diet pairs in *T. commodus*. In each panel, lines represent the response of a given genotype across two diet pairs.

Figures

Fig. 1

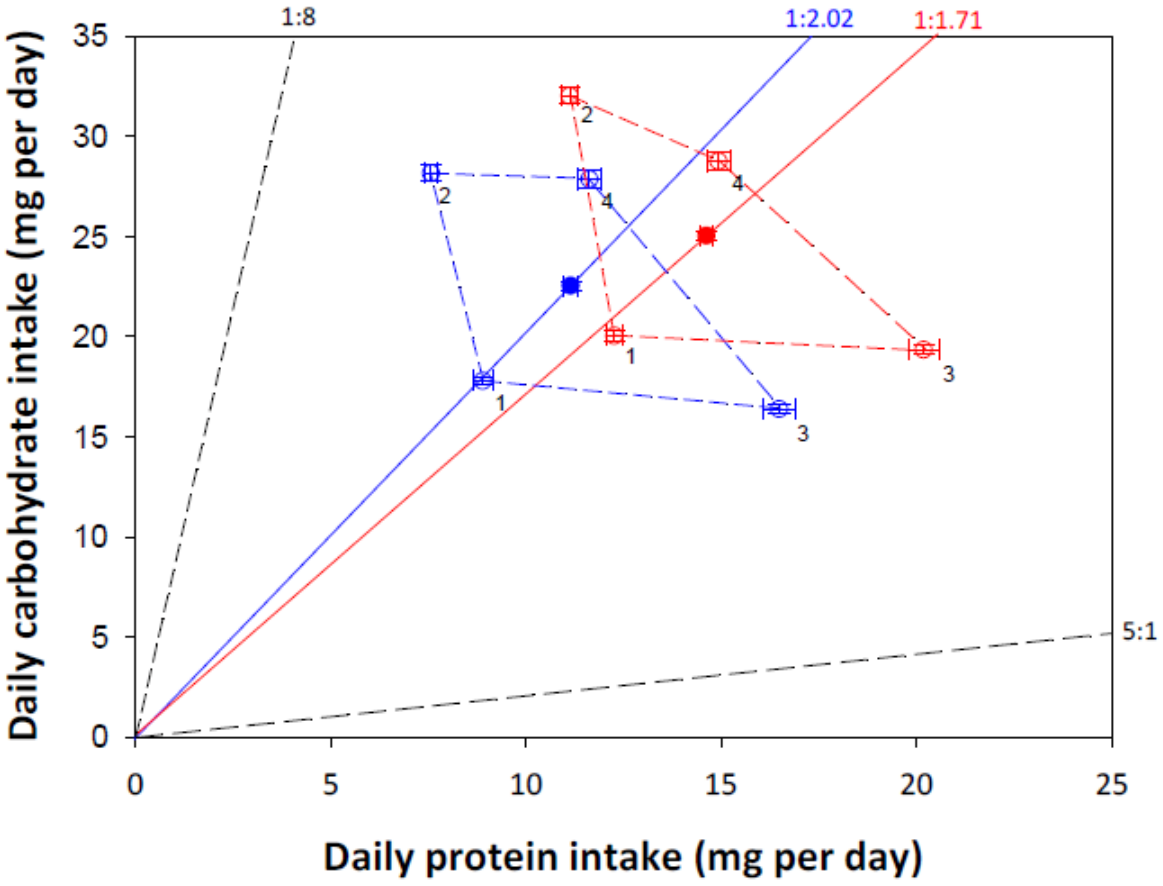


Fig. 2

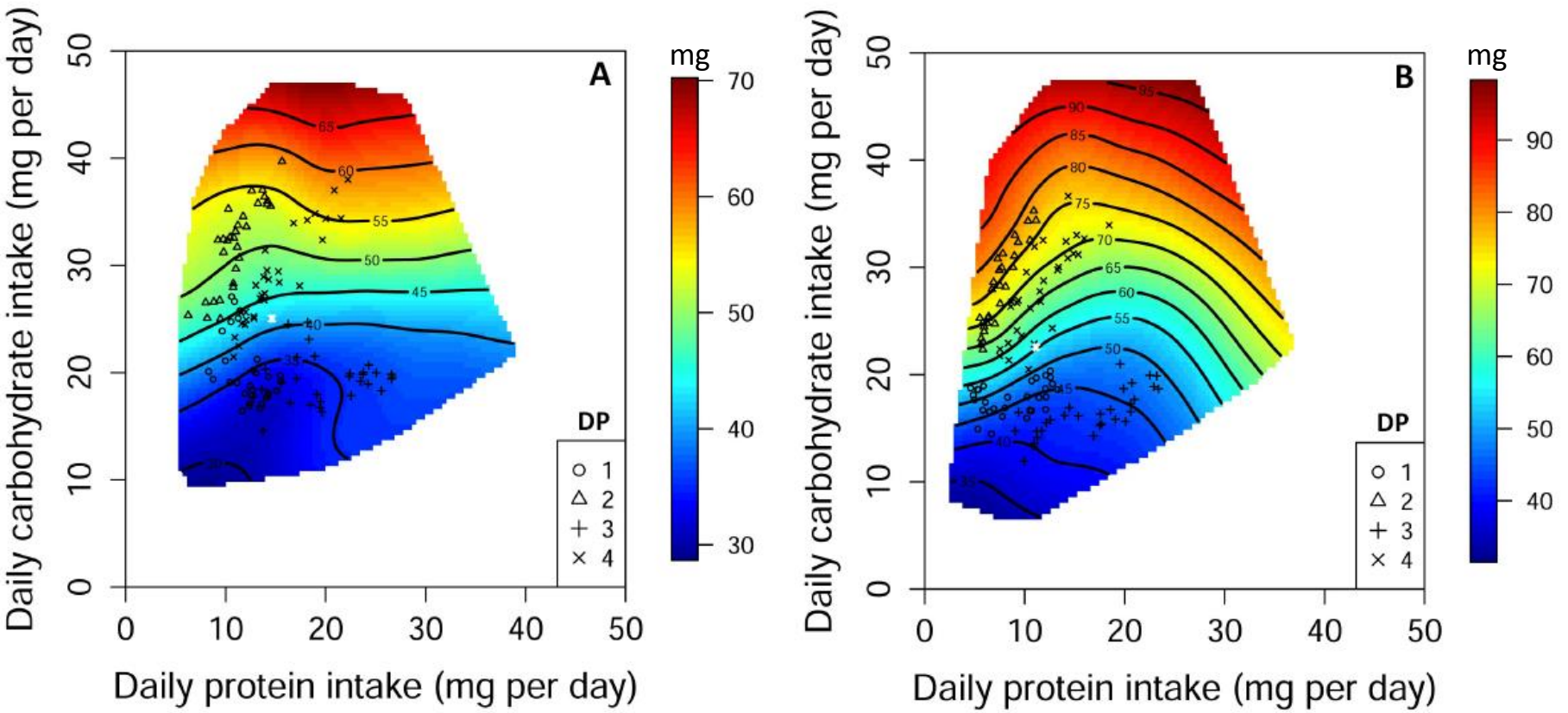

A
B
mg
DP
1
2
3
4
Daily carbohydrate intake (mg per day)
Daily protein intake (mg per day)
0
10
20
30
40
50
30
40
50
60
70
80
90

Fig. 3

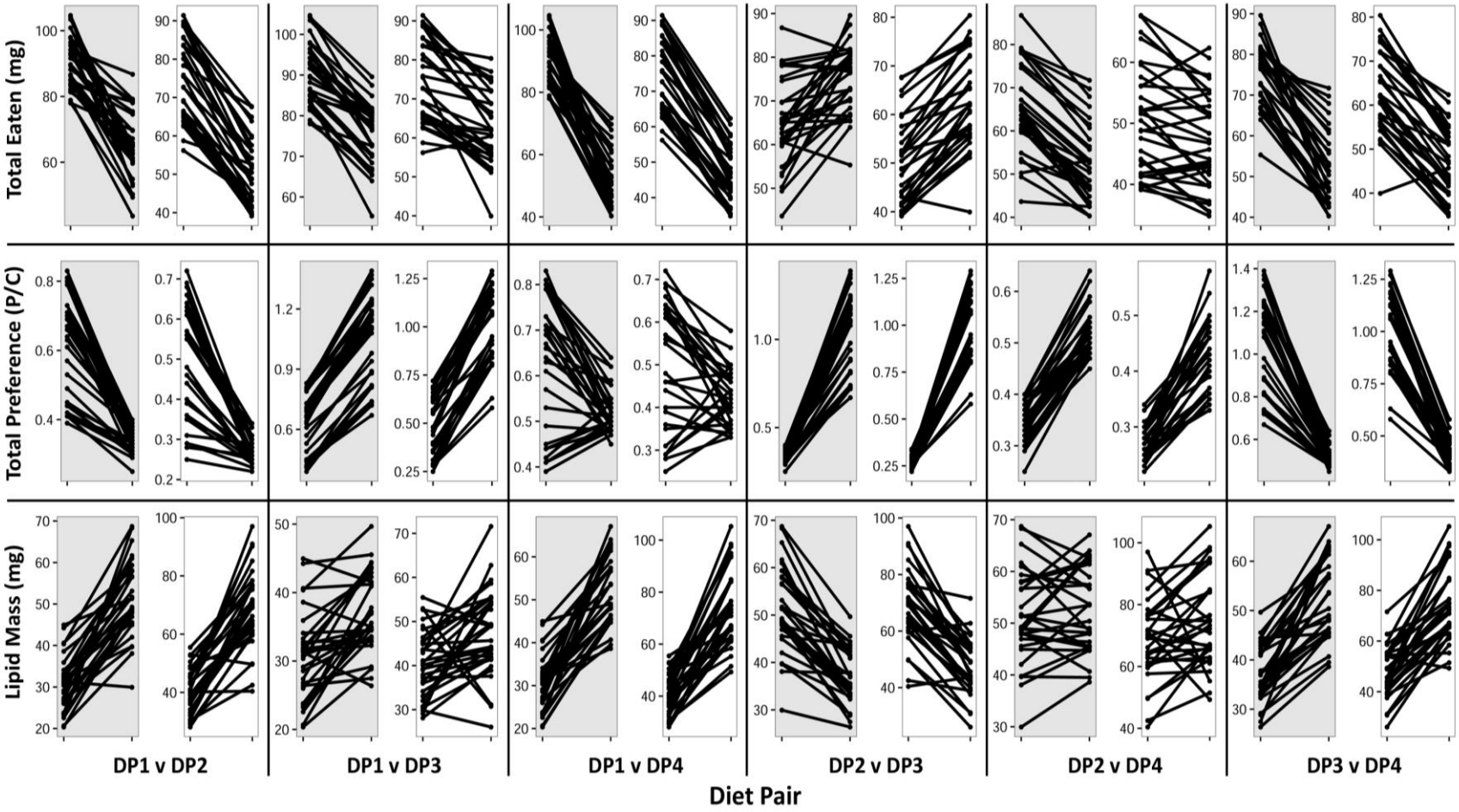
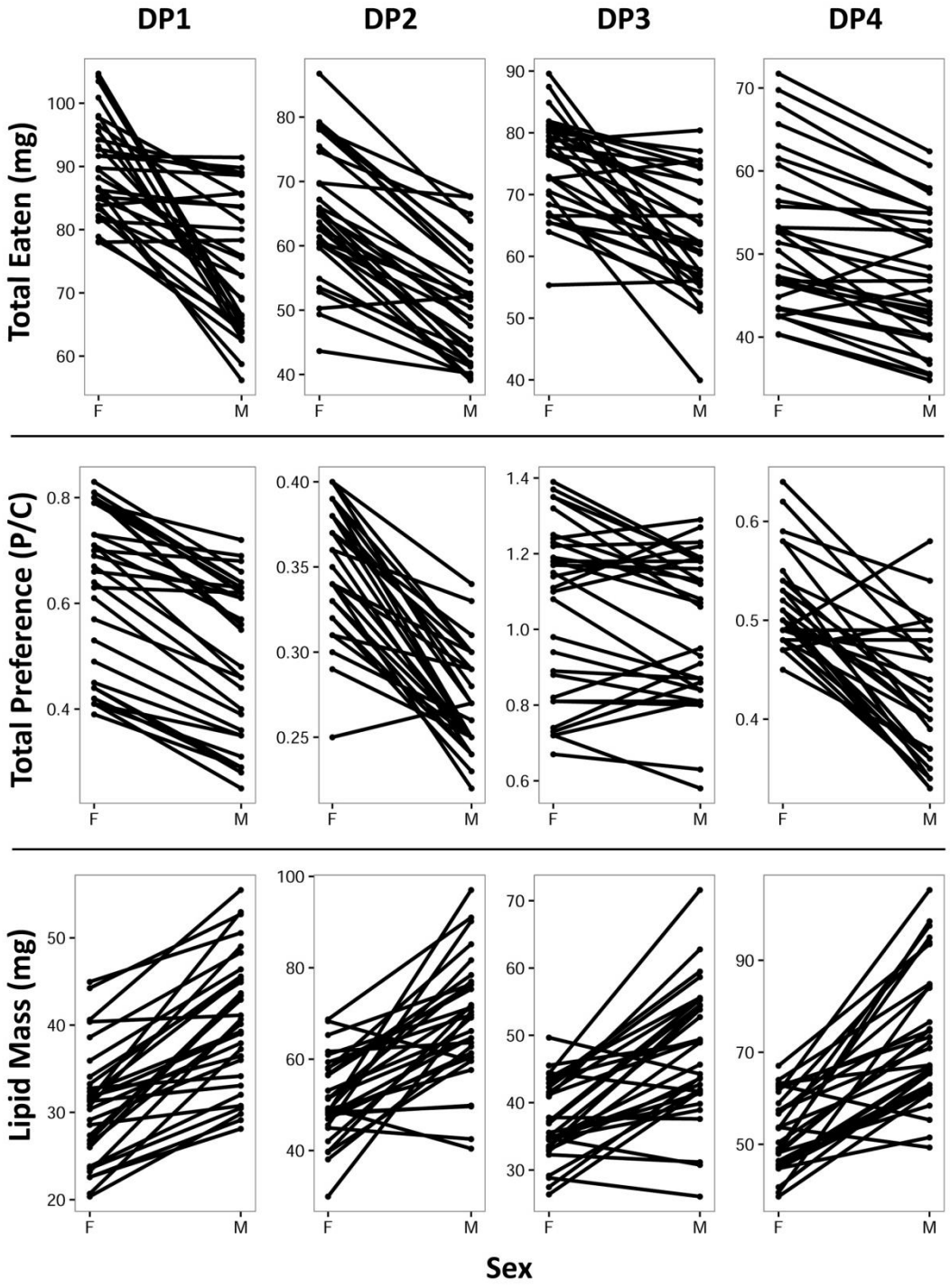

Total Eaten (mg)
Total Preference (P/C)
Lipid Mass (mg)
DP1 v DP2
DP1 v DP3
DP1 v DP4
DP2 v DP3
DP2 v DP4
DP3 v DP4
Diet Pair

Fig. 4


DP1
DP2
DP3
DP4
Total Eaten (mg)
Total Preference (P/C)
Lipid Mass (mg)
F
M
Sex