

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

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24 **Abstract**

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

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

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54 Introduction

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

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

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

88 Despite this evidence for active nutrient regulation by animals, very little is known
89 about the background genetic architecture that controls nutrient intake. There is support
90 from studies on humans and rodent models that macronutrient intake has a genetic basis
91 (Liu and Lloyd, 2013) and some evidence for genetic variation over food intake in *D.*
92 *melanogaster* (Reddiex *et al.*, 2013; Garlapow *et al.*, 2015). Furthermore, a number of
93 studies have identified candidate genes affecting variance over food intake with phenotypic
94 variation over food intake. This variation was due to multiple segregating loci with alleles
95 sensitive to environmental effects (Falconer and Mackay, 1996; Garlapow *et al.*, 2015,
96 2016), indicative of interactions between genes and the environment.

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

117 2010), with examples found in a number of taxa (e.g. spider monkey (*Ateles chamek*) (Felton
118 *et al.*, 2009); humans (Gosby *et al.*, 2011, 2014; Martens *et al.*, 2013); mice (Sørensen *et al.*,
119 2008)).

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

134 In addition to GxEs over dietary intake and fat deposition, one must also take into
135 account the different nutritional requirements of males and females (Maklakov *et al.*, 2008;
136 Simpson and Raubenheimer, 2012; Harrison *et al.*, 2014; Jensen *et al.*, 2015) resulting from
137 different reproductive strategies. In most sexually reproducing species, males typically
138 allocate more resources to mate competition while females typically allocate more
139 resources to offspring production (Trivers, 1972). This results in males and females having
140 different nutritional requirements, and drives the evolution of sex-specific nutritional
141 optima for reproduction (e.g. *D.melanogaster* (Jensen *et al.*, 2015); *T.commodus* (Maklakov
142 *et al.*, 2008)). Furthermore, sexual selection can promote sexual divergence in the strength
143 and direction of nutritional trade-offs between various life-history traits, for example
144 lifespan and reproduction (Lee *et al.*, 2008; Maklakov *et al.*, 2008; Reddiex *et al.*, 2013;
145 Jensen *et al.*, 2015). How males and females actively regulate their intake of nutrients will
146 determine the optimal expression of multiple fitness related traits (Simpson and
147 Raubenheimer, 2012) and may also influence the interactions between genotype and the
148 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 $28^{\circ}\text{C} \pm 1^{\circ}\text{C}$, 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*

180 Using the protocol established in South et al (South *et al.*, 2011) we made four
181 powdered, holidic (i.e. chemically defined) diets. These four diets were used to make four
182 dietary pairs, with each pair containing one diet with a P:C ratio of 1:8 and one with a P:C
183 ratio of 5:1. We provided these diets in one of two nutritional dilutions (%P+C content), 36%
184 or 84%. The four diet pairs (DPs) are as follows: DP1: 1:8 (36%) versus 5:1 (36%), DP2: 1:8
185 (84%) versus 5:1 (36%), DP3: 1:8 (36%) versus 5:1 (84%), and DP4: 1:8 (84%) versus 5:1
186 (84%) with composition also provided in Table S1. These diets were selected from a larger
187 geometric array of a possible 24 diets because they provide a broad coverage of potential
188 nutrient space (Figure S1) and have been used in previous choice feeding experiments
189 (South *et al.*, 2011; Bunning *et al.*, 2015).

190

191 *Quantitative Genetic Breeding Design*

192 To estimate the quantitative genetics of total diet eaten, nutritional preference and
193 lipid mass, we used a split-brood half-sib breeding design whereby sons and daughters from
194 each full-sib family were split across four different diet pairs and their intake of nutrients
195 measured under dietary choice for 21 days. The half-sib breeding design was established by
196 mating each of 30 randomly chosen virgin sires with three randomly chosen dams. A total of
197 50 offspring from each dam were collected and reared in a family group in an individual
198 plastic container (10 x 10 x 5cm) for three weeks, with access to an *ad libitum* supply of
199 ground cat food (Purina Go Cat Senior[®], St Louis, MO, USA) and water provided in a 5cm
200 plastic tube plugged with cotton wool. After three weeks, 12 sons and 12 daughters per dam
201 were isolated and established at random in individual plastic containers (5cm x 5cm x 5cm)
202 and provided with *ad libitum* cat food pellets and water, and checked daily for eclosion to
203 adulthood. Containers were cleaned and fresh food and water were provided weekly. On
204 the day of eclosion, we randomly allocated 3 sons and 3 daughters per dam to each of four
205 diet pairs (total $n = 1080$ sons and 1080 daughters; see Fig S2 for a graphical representation
206 of our breeding design). Fresh diet was provided every three days for a total of 21 days (i.e.
207 a total of seven feeding periods). Experimental animals were mated with a stock animal of
208 the opposite sex on the evening of day 8 post-eclosion and removed on day nine with
209 females provided with a petri dish of moist sand thereafter for oviposition.

210

211 *Feeding Regime*

212 Experimental feeding followed established protocols used previously (South *et al.*,
213 2011). In brief, two dishes of diet of measured dry weight were provided to each cricket
214 according to assigned diet pair. Food was provided in feeding platforms constructed by
215 gluing the upturned lid of a vial (1.6 cm diameter, 1.6cm deep) onto the middle of a petri
216 dish (5.5 cm diameter) and water was provided *ad libitum* in a 5ml test tube plugged with
217 cotton wool. Any diet spilled during feeding was collected in the petri dish and weighed. All
218 diets were dried in an oven (Binder FD115, Germany) at 30°C for 72 hrs before weighing.
219 Feeding platforms were weighed before and after each feeding period using an electronic
220 balance (Ohaus Explorer Professional EP214C, Switzerland). Faeces were removed from the
221 diet and feeding platform using forceps prior to re-weighing. Diet consumption was
222 calculated as the difference in dry weight of diet before and after feeding. This amount of
223 consumed diet was converted to a weight of P and C ingested by multiplying by the
224 proportion of these nutrients in the diet (South *et al.*, 2011).

225

226 *Measuring Lipid Mass*

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

236

237 *Statistical Analysis*

238 Quantitative genetic analyses were performed using animal models fitted in ASReml
239 (version 3) (Gilmour *et al.*, 2009). An animal model is a form of linear mixed-effect model
240 incorporating pedigree information where an individual's genetic merit is included as a
241 random effect allowing for the estimation of the additive genetic (co)variance (Wilson *et al.*,
242 2010). We examined three phenotypic traits: the total amount of diet eaten (TE) (including
243 nutritional and non-nutritional components), total nutritional preference (TP) (calculated as

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

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

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

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

280

281 **Results**

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

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

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

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

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

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

349

350 **Discussion**

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

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

385 Optimal foraging theory (Stephens and Krebs, 1986) predicts that when in a
386 nutritionally imbalanced environment, an animal may actively regulate their intake of
387 nutrients either through compensatory feeding or by eating non-randomly from multiple
388 food sources (Simpson and Raubenheimer, 2012). Our finding that there is considerable
389 variation in both TE and TP across DPs and the sexes suggests that both processes are
390 operating in male and female *T. commodus* but to differing degrees. We found that both
391 sexes increased the total amount of diet they consumed on the lowest nutrition pair (DP1,
392 36% nutrition) compared to highest nutrition pair (DP4, 85%) but this increase was larger in
393 females (72%) than males (52%). While compensatory feeding has been demonstrated in a
394 variety of animal taxa (Simpson & Raubenheimer 2012), only a few studies have reported
395 sex differences in this behaviour and existing studies show that the magnitude of
396 compensatory feeding is higher in males than in females (Barreto *et al.*, 2003). We also
397 show that females have consistently higher TP values than males on each DP and although
398 both sexes show an overall preference for C intake over P intake, the RIP was relatively
399 more P biased in females (P:C ratio = 1:1.71) than males (P:C ratio = 1:2.02). This contrasts
400 with earlier work in *T.commodus* that showed no sex-differences in the regulated intake of P
401 and C (Maklakov *et al.*, 2008). The differences we observe in *T. commodus*, however, can be

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

417 Current theories on the link between diet and obesity have highlighted the over
418 ingestion of energy dense foods as a primary factor in weight gain (Mathes *et al.*, 2011;
419 Raubenheimer *et al.*, 2015). While we cannot show 'over-ingestion' in our study, we do
420 show that lipid deposition in male and female *T. commodus* was significantly greater on the
421 DP with the highest total nutrition (DP4, 84% nutrition) and lowest on the DP containing
422 lowest total nutrition (DP1, 36% nutrition). However, we also show that lipid deposition is
423 not only contingent on the energy (caloric) content of the diet but also the relative intake of
424 nutrients. This is illustrated by the difference in lipid deposition of both sexes when feeding
425 from DP2 and DP3; both DPs contain the same total energy content, but the highest nutrient
426 diet in DP2 is C biased (P:C = 1:8, 84% total nutrition) whereas it is P biased on DP3 (P:C =
427 5:1, 84% total nutrition). Consequently, the significantly higher lipid deposition of males and
428 females feeding from DP2 than DP3 suggests that the intake of C is more important to lipid
429 deposition than P intake. Our response surface analysis also shows that LM was maximised
430 in both sexes at a high intake of C and low intake of P (Table 2, Fig. 2). This finding supports
431 the well-established link between increased C intake and lipid deposition reported in a
432 range of animal taxa (Mathes *et al.*, 2011; Raubenheimer *et al.*, 2015). It also explains the
433 lower LM of females than males on each of the DPs, despite their higher overall

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

448 The physiological systems that control lipid deposition rely on a highly complex,
449 polygenic contribution of genes. There exist examples from a number of classic molecular
450 genetic studies using mice (Marie *et al.*, 2000) and human models (Raubenheimer *et al.*,
451 2015) but there is also growing evidence using more recent genomic approaches in humans
452 (e.g. Robbins and Savage, 2015) and *C.elegans* (e.g. Zhang *et al.*, 2010). Further studies have
453 also specifically looked at the genetic components of lipid acquisition, storage and
454 mobilisation in five insect species (*D.melanogaster*, mosquitoes (*Anopheles gambiae*), honey
455 bees (*Apis mellifera*), moths (*Bombyx mori*), and beetles (*Tribolium castaneum*) (Horne *et*
456 *al.*, 2009), and between dimorphic wing morphs in the cricket *G.firmus* (e.g. Schilder *et al.*,
457 2011; Nanoth Vellichirammal *et al.*, 2014). The complexity surrounding lipid deposition is
458 perhaps not surprising given that lipid deposition (and by extension obesity) is influenced by
459 interactions between many variables, for example; environment (dietary and social) (Qi and
460 Cho, 2008; Mathes *et al.*, 2011), microbiota (Schilder and Marden, 2006; Wolf and Lorenz,
461 2012), various life-history traits including reproduction and ageing (Hansen *et al.*, 2013) and
462 other genes either related to feeding behaviour and lipid deposition (e.g. “thrifty gene
463 hypothesis”) (Neel, 1962; Barsh *et al.*, 2000) and/or genetic pathways linked to other life-
464 history traits (e.g. Insulin-like growth factor-1 (IGF-1) (Post and Tatar, 2016); mechanistic

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

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

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

496 Secondly, there were consistent positive genetic correlations between TE and TP
497 across all DPs in males and also in DP2 for females. In our study, TP was measured as the
498 total intake of P divided by the total intake of C. Higher values of TP, therefore, mean a
499 preference for more P relative to C, even when there is an absolute preference for C ($TP <$
500 1.0 , and shown in DP1, 2 and 3 of Fig S2). Consequently, this positive genetic correlation
501 indicates that in males and in some nutritional environments for females, the genes that
502 govern the preference for P relative to C, are positively associated with the genes for dietary
503 consumption. Finally, there were negative genetic correlations between TP and LM on DP1,
504 3 and 4 in males and DP1 in females. This indicates that the genes for LM are negatively
505 associated with those governing the preference for P relative to C. Collectively, both of
506 these patterns of additive genetic covariance between traits provide partial support for the
507 PLH at the genetic level. The PLH predicts that in a nutritionally imbalanced environment
508 where P is limited, the powerful P appetite will stimulate individuals to increase their dietary
509 consumption in an attempt to gain more P (Simpson and Raubenheimer, 2005; Sørensen *et al.*,
510 *2008*; Gosby *et al.*, 2014), a pattern that is supported by the positive genetic correlation
511 between TP and TE, where a preference to consume P is causing an increase in the TE, for
512 example males in DP2 have a genetic correlation of $0.93 (\pm 0.16)$ between TE and TP. DP2 is
513 highly carbohydrate biased and so males seeking to increasing their P intake are consuming
514 increasing amounts of the available diets. Furthermore, the PLH predicts that a side effect of
515 attempting to consume a limited supply of P is the over-ingestion of more abundant
516 nutrients (such as C) that cause increased lipid deposition and predispose an individual to
517 obesity (Simpson and Raubenheimer, 2005; Sørensen *et al.*, 2008; Gosby *et al.*, 2014). The
518 observed negative genetic correlations between TP and LM agree with this prediction,
519 although it also supports the alternate view that the genes for C preference are directly
520 linked to those for LM. Further support for this prediction would have come from positive
521 genetic correlations between TE and LM, however, this relationship was inconsistent in
522 males being negative in DP1 and positive in DP3.

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

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

545

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550

551 **Data Accessibility.** All data will be deposited at dryad (www.datadryad.org) upon
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553

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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.

	<i>F</i>	<i>df</i>	<i>P</i>
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.

Sex	Linear effects		Nonlinear effects		
	P	C	P x P	C x C	P x C
Males					
Gradient \pm SE	-0.08 \pm 0.03	0.52 \pm 0.03	0.09 \pm 0.02	-0.00 \pm 0.02	-0.10 \pm 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 \pm SE	-0.03 \pm 0.03	0.49 \pm 0.03	0.04 \pm 0.02	0.02 \pm 0.03	-0.00 \pm 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 (\pm 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. Reaction 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

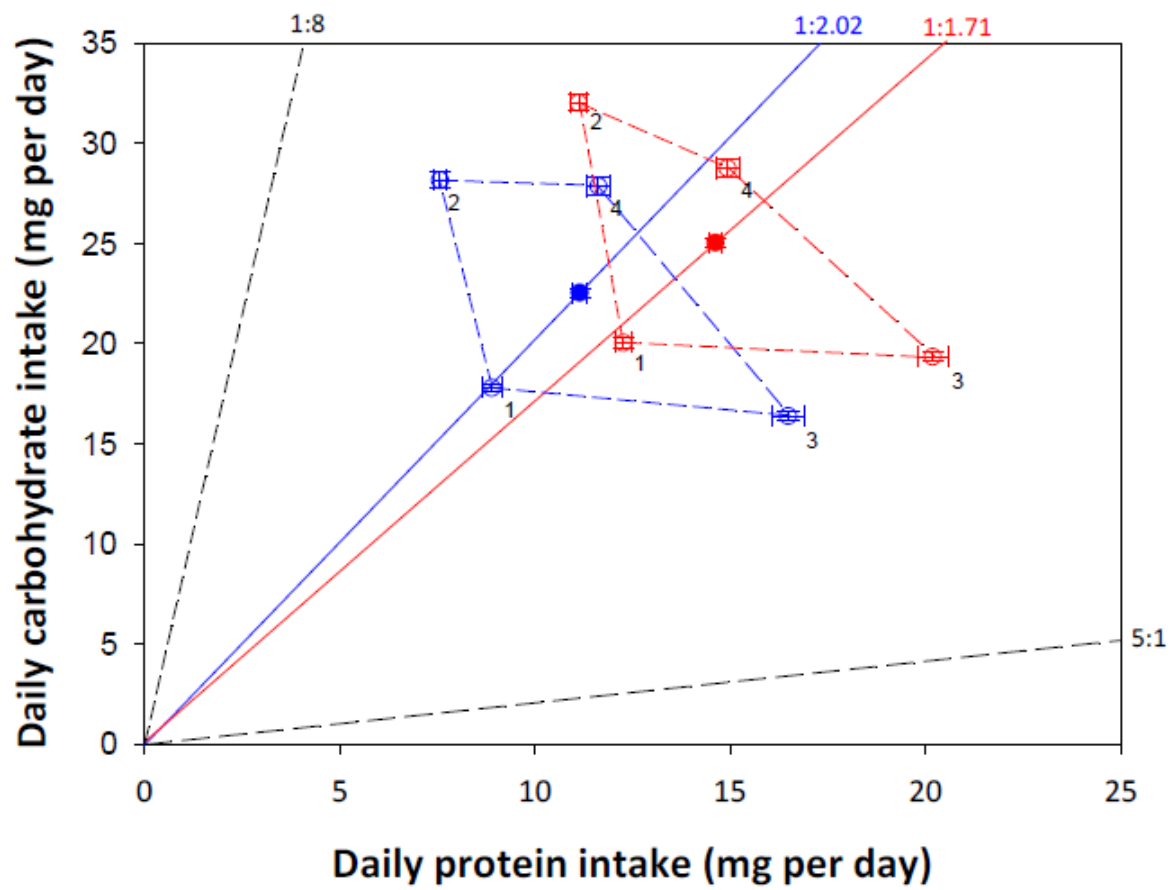


Fig. 2

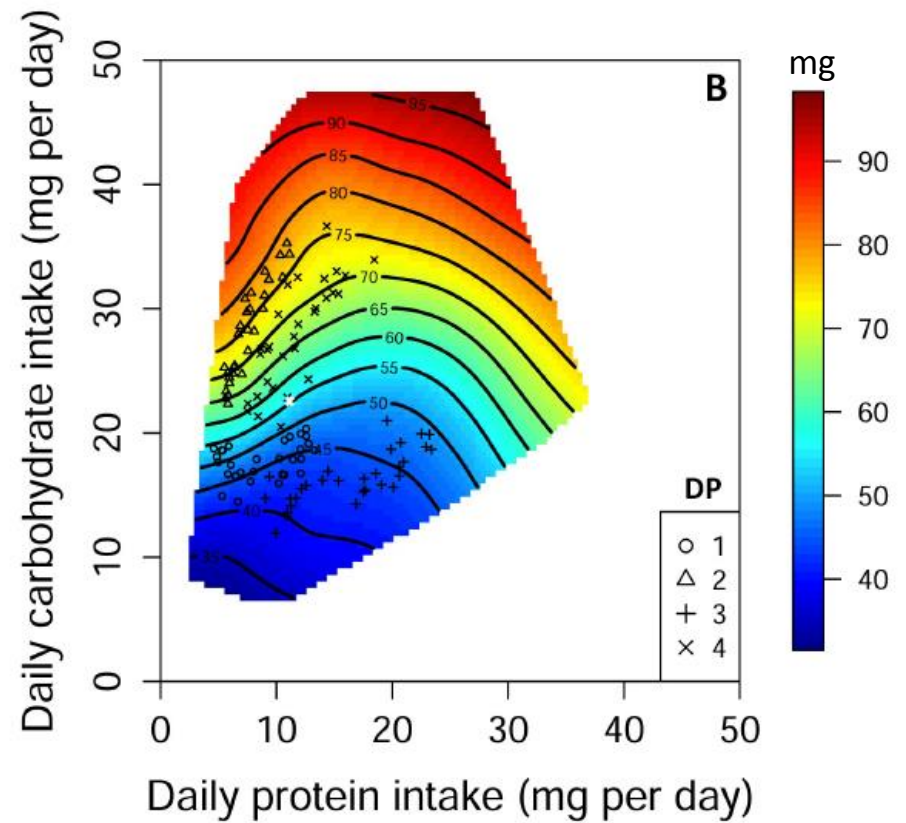
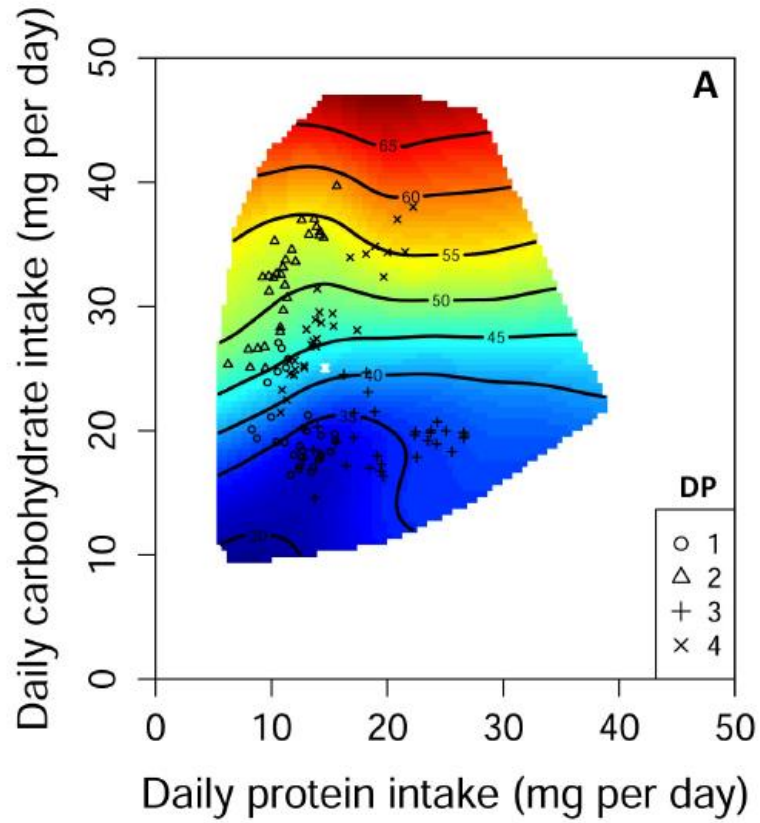


Fig. 3

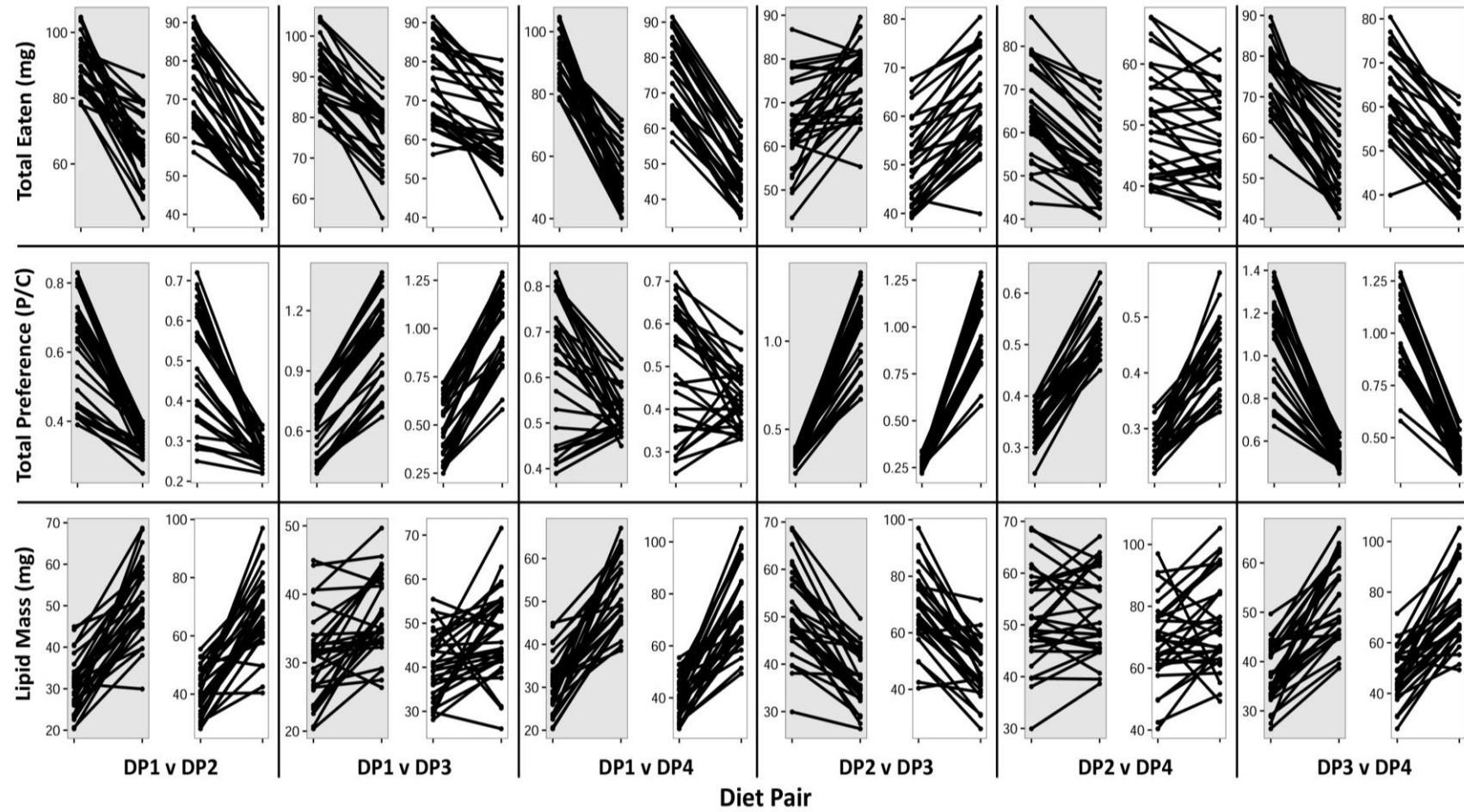


Fig. 4

