RESEARCH ARTICLE

Geometric analysis of macronutrient selection in the adult domestic cat, Felis catus

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SUMMARY

We report feeding studies on adult domestic cats designed to disentangle the complex interactions among dietary protein, fat and carbohydrate in the control of intake. Using geometric techniques that combine mixture triangles and intake plots from the geometric framework, we: (1) demonstrate that cats balance their macronutrient intake, (2) estimate the composition of the target balance and (3) reveal the priorities given to different macronutrients under dietary conditions where the target is unachievable. Our analysis indicates that cats have a ceiling for carbohydrate intake, which limits ingestion and constrains them to deficits in protein and fat intake (relative to their target) on high-carbohydrate foods. Finally, we reanalyse data from a previous experiment that claimed that kittens failed to regulate protein intake, and show that, in fact, they did. These results not only add to the growing appreciation that carnivores, like herbivores and omnivores, regulate macronutrient intake, they also have important implications for designing feeding regimens for companion animals.

Key words: macronutrient regulation, geometric framework, carnivore nutrition, predation, domestic cat.

INTRODUCTION

Establishing the nutritional priorities that govern foraging behaviour, diet selection and post-ingestive processing is fundamental to predicting an animal’s interactions with its nutritional environment, with consequences that extend from the health and evolutionary fitness of the individual to the structuring of populations and communities (Raubenheimer et al., 2009; Simpson et al., 2009; Kearney et al., 2010). Conventional models have assumed that animals typically prioritise a single food component, usually energy, nitrogen or toxins. However, experimental studies on herbivores and omnivores across a wide range of taxa have shown that animal nutrition can be better understood in terms of balancing multiple nutritional and non-nutritional components of foods (Belovsky, 1984; Kyriazakis and Emmans, 1991; Kyriazakis et al., 1991; Raubenheimer and Simpson, 1997; Cheng et al., 2008; Felton et al., 2009). This is predictable in theory because such animals experience a range of food types and qualities, in which nutritional components need not be tightly correlated, necessitating separate regulation of key nutrient dimensions. In contrast, the prevailing view until recently was that predators have no need to regulate intake of multiple nutrients, but are mainly limited by food availability (Westoby, 1978; Fryxell and Lundberg, 1997). This assumption is founded on the premise that predators feed on relatively rare, high-quality foods that are less variable in composition than the foods of herbivores and omnivores (Slansky and Scriber, 1985; Galef, 1996). In recent years, however, this view of predator nutritional ecology has been called into question (see Mayntz et al., 2009).

Establishing whether predators can regulate their intake of multiple nutrients is a fundamental issue for nutritional ecology (Raubenheimer et al., 2009), but it is especially important to understand the regulatory capacities of domestic pets such as cats, as the diet of the animal is largely determined by the carer. Providing inappropriate diet compositions has implications for animal health and welfare, and potentially also for urban ecology through pets supplementing their diet from nature. There are additional implications for understanding the evolution of nutritional biology under artificial selection.

Results from earlier studies on the domestic cat, Felis catus, have been ambiguous. Cook et al. claimed lack of regulation of protein intake in cats offered pairwise combinations of foods differing in protein content (Cook et al., 1985). In contrast, cats were reported to distinguish between foods based on the concentration of methionine (Rogers et al., 2004). Bradshaw concluded that cats are unlikely to show nutrient-specific food selection, but instead use general mechanisms, such as neophilia and neophobia, to avoid nutritional imbalances (Bradshaw, 2006).

In contrast, there is no ambiguity in the results of recent experiments, which have unequivocally demonstrated that predators, including invertebrates (Mayntz et al., 2005; Raubenheimer et al., 2007), fish (Sanchez-Vazquez et al., 1999; Rubio et al., 2003) and mink (Mayntz et al., 2009), do in fact regulate and balance their intake of multiple nutrients, notably protein and lipid.

Here we describe an extensive series of dietary studies on the domestic cat, based on geometric multivariate analysis of the interactions between protein, fat and carbohydrate. Most domestic cats are fed commercial pet foods by their owners. Some of these products are moist and others are based on a dry formulation. As well as differing in water content and texture, there are macronutritional differences between wet and dry commercial products (Raubenheimer et al., 2009), but these have not been experimentally tested for domestic cats.
foods, notably a higher carbohydrate content of dry foods (required for their manufacture). We have therefore built into the experimental designs an investigation of the nutritional consequences of these different food types. Our results show strong nutritional regulation, reinforcing the fact that macronutrient regulation is common across trophic levels and providing important information for the design of domestic cat nutritional regimes.

MATERIALS AND METHODS

Animals, diet composition and general protocols

Adult, neutered domestic short hair cats (*Felis catus* Linnaeus 1758) of both sexes bred and housed at the WALTHAM® Centre for Pet Nutrition (WCPN), Melton Mowbray, UK, were used in these experiments. Throughout each experiment (except Expt 9, as explained below), the cats were housed and fed individually in purpose-built, behaviourally enriched lodges (1.1 x 2.5 x 2.1 m width x depth x height) and were socialised as a group for ~1 h each day and had access to drinking water at all times. For Expt 9, the individual housing lodges were not available and so cats were housed in groups of 12 and fed in two 1 h periods each day in individual feeding stations (plastic boxes measuring 0.5 x 0.5 x 0.5 m). The cats were housed in social groups when not participating in experiments. The studies were approved by the WCPN Ethical Review Committee.

Six dry-format diets were manufactured using standard processing (extrusion) conditions at Mars Petcare, Verden, Germany. These diets were formulated based on Mars Inc. commercial recipes with the inclusion level of poultry meal, maize gluten, ground rice, wheat flour and beef tallow altered to achieve differences in the macronutrient energy ratios of the diets (Table 1). Six wet-format diets were manufactured using standard processing (canning) conditions at Mars Petcare, Saint Denis de l’Hôtel, France. These diets were formulated based on Mars Inc. commercial recipes with the inclusion level of chicken breast, soya protein isolate, lard and wheat flour altered to achieve differences in the macronutrient energy ratios of the diets (Table 2). Both wet and dry diets were formulated to meet the National Research Council and Association of American Feed Control Officials (AAFCO) guidelines for adult cats.

Table 1. Macronutrient compositions of the diets used in the dry diet experiments

| Experiment | Diet  | Predicted metabolisable energy* (MJ kg⁻¹) | PER (%) | FER (%) | CER (%) |
|------------|------|------------------------------------------|---------|---------|---------|
| 1          | pIC¹ | 14.39                                    | 26      | 22      | 52      |
|            | Pc   | 14.07                                    | 51      | 23      | 26      |
|            | pFc  | 16.91                                    | 27      | 45      | 28      |
| 2          | Pc   | 14.46                                    | 48      | 26      | 28      |
|            | pFc  | 16.35                                    | 22      | 53      | 28      |
|            | gfc  | 17.86                                    | 34      | 42      | 24      |
| 3          | pIC² | 14.43                                    | 21      | 23      | 56      |
|            | pfc  | 16.34                                    | 18      | 39      | 43      |
|            | pFc  | 18.21                                    | 17      | 52      | 31      |
| 4          | pIC  | 14.55                                    | 24      | 25      | 51      |
|            | Pc   | 13.92                                    | 49      | 23      | 28      |
|            | pFc  | 14.06                                    | 39      | 24      | 37      |
| 5          | pFc  | 14.55                                    | 24      | 25      | 51      |
|            | Pc   | 13.92                                    | 49      | 23      | 28      |

CER, carbohydrate energy ratio; FER, fat energy ratio; PER, protein energy ratio.

*Proximate analysis and modified Atwater factors (protein, 16.44 kJ g⁻¹; fat, 35.66 kJ g⁻¹; digestible CHO, 16.44 kJ g⁻¹) were used to calculate the predicted metabolisable energy of each diet.

*Where diets with the same letter descriptors have different energy content and macronutrient profiles, this is due to analytical differences between batches of diet made to the same recipe.

See Materials and methods for diet descriptions.

Table 2. Macronutrient compositions of the diets used in wet diet experiments

| Experiment | Diet  | Predicted metabolisable energy* (MJ kg⁻¹) | PER (%) | FER (%) | CER (%) |
|------------|------|------------------------------------------|---------|---------|---------|
| 6          | pIC  | 3.07                                     | 41      | 27      | 31      |
|            | pFc  | 3.88                                     | 39      | 54      | 8       |
|            | pFc  | 3.32                                     | 40      | 46      | 14      |
| 7          | pIC  | 3.07                                     | 41      | 27      | 31      |
|            | Pc   | 3.19                                     | 68      | 30      | 3       |
|            | pFc  | 3.08                                     | 53      | 32      | 15      |
| 8          | Pc   | 3.19                                     | 68      | 30      | 3       |
|            | pFc  | 3.88                                     | 39      | 54      | 8       |
|            | gfc  | 3.36                                     | 53      | 44      | 3       |
| 9          | pIC² | 2.88                                     | 40      | 28      | 32      |
|            | Pc   | 3.39                                     | 67      | 31      | 2       |
|            | pFc  | 3.49                                     | 41      | 57      | 2       |

*Proximate analysis and modified Atwater factors (protein, 16.32 kJ g⁻¹; fat, 32.22 kJ g⁻¹; digestible CHO, 12.55 kJ g⁻¹) were used to calculate the predicted metabolisable energy of each diet.

*Where diets with the same letter descriptors have different energy content and macronutrient profiles, this is due to analytical differences between batches of diet made to the same recipe.

See Materials and methods for diet descriptions.
feline maintenance through addition of vitamin and mineral mixes, taurine and L-methionine.

Detailed experimental designs are given below, but in general, for dry diet experiments each bowl contained 150 g of the allocated diet and the food was available to the cats for 22 h, from 10:30 to 08:30 h the following morning, at which time any uneaten food was weighed to allow calculation of the amount of each diet eaten. For wet diet experiments, each bowl contained 190 g of the allocated diet from 10:30 to 15:00 h and was replaced with a fresh aliquot (190 g) from 15:00 to 08:30 h the next day. Any uneaten food was weighed as food was collected at 15:00 and 08:30 h.

Dry diet experiments

Only cats that had been fed on dry food from weaning were used in these experiments. At the time these experiments were performed there were only 24 cats at WCPN that had been maintained on dry food since weaning. Therefore, some cats took part in more than one experiment. Eleven cats were unique to Expt 1 and one cat took part in Expts 1 and 4. Another group of 11 cats were common to Expts 2, 3 and 4; the same 12 cats were used in Expts 2 and 3. The 12 cats in Expt 5 had previously taken part in Expts 1 (three cats) and 2 (nine cats).

Experiment 1. Variable protein, carbohydrate and fat

Twelve neutered adult cats (11 female, one male) were allocated to this experiment, although two female cats were removed because of low food intake and loss of body mass; these data were excluded from analysis. Cats (N=10) were aged 3.1 to 6.1 years (mean ± SEM=3.6±0.3 years) and weighed 4.49±0.26 kg at the start of the experiment. The experiment had three phases. In this experimental design, the cats in all three phases could theoretically achieve any diet composition that fell within the region of composition space that is delineated by the triangle in Fig. 1A.

Phase 1: naive simultaneous self-selection

For 7 days, cats were given three foods simultaneously in three separate bowls from which to self-select a diet: high fat (pFc), high carbohydrate (pfC) and high protein (Pfc). To avoid positional bias, the position of each diet was rotated daily. The proportional protein:carbohydrate:fat compositions of the foods are shown in Fig. 1A.

Phase 2: monadic diets, sequential self-selection

Cats were cycled through eight 3-day periods during which they were confined to a different food (pFc, pfC or Pfc) on each of the three

Fig. 1. Results from Expt 1, dry diets with variable protein, carbohydrate and fat content. Green circles, mean diet composition (A) and nutrient intakes (B–D) of naive simultaneous self-selecting cats (phase 1); blue circles, the same results for sequential self-selection (phase 2); red circles, the same results for experienced self-selection (phase 3). The region of accessible composition space is indicated by shading in A. The solid black lines in B–D represent the two-dimensional nutrient balance of the experimental foods, and hence the trajectory to which the cats were confined when eating the respective foods (i.e. nutrient rails). The small black circles show the mean daily intakes of cats confined to the respective foods during the sequential self-selecting stage of the experiment (phase 2). The intake of each diet was averaged for individual cats across all eight cycles of the phase, and the means of these means are plotted in the figure. Therefore, each cat is represented only once for a given diet, and each appears in the means for all three diets. The dashed blue line shows the intake trajectory compounded over all eight 3-day cycles and all experimental replicates in the sequential self-selection phase (phase 2) of the experiment. The aim of including this is to show the mean relative step size and the contribution it makes to overall nutrient intake by sequential self-selecting cats (blue circles). By convention we have represented the food with the steepest, shallowest and intermediate nutrient rails, respectively, as the sequence of intakes, but in reality the sequence differed among cats. See Materials and methods for diet descriptions. Values in B–D are means ± s.e.m.; N=10 cats.
days. To reduce sequence effects, cats were randomly assigned to one of six orders of diet presentation. They were therefore unable to self-select a diet within each day, but could regulate their intake on successive days so as to compensate for imbalances accrued over previous days. This was, therefore, a sequential self-selection design in which the switching interval (1 day) was experimentally regulated. Phase 2 also served as a conditioning phase in which the cats gained experience of each of the foods separately.

Phase 3: experienced simultaneous self-selection
In this phase, the regime of Phase 1 was repeated (simultaneous self-selection) on the now ‘experienced’ cats.

Experiment 2. Fixed carbohydrate
Twelve neutered adult female cats aged 2.0 to 4.1 years (mean ± s.e.m.=3.5±0.2 years) and weighing 5.04±0.16 kg participated in this experiment. The design and feeding regimen of this experiment were the same as Expt 1, except here the high-carbohydrate food (pFc) was replaced by pfC food, which had the same level of carbohydrate as Pfc and pFc, but a protein:fat ratio intermediate between these foods. The point representing this food in mixture space lies immediately to the left of the label pfC but is obscured by the blue dot in Fig.2A. In this experimental design, the diets self-selected in all three phases were constrained to lie within the narrow grey region delineated by the lines joining the food composition points, rather than the larger triangle of Expt 1.

Experiment 3. Fixed protein
Twelve neutered adult female cats were used, although one was removed because of low food intake and loss of body mass, and the data were excluded from analysis. Cats (N=11) were aged 2.3 to 4.4 years (mean ± s.e.m.=3.7±0.2 years) and weighed 5.03±0.19 kg at the start of the study. The design of this experiment was the same as Expt 2, except that the three experimental foods had equal protein density but differed in their fat:carbohydrate ratio. Food compositions are shown by the black dots in Fig.3A.

Experiment 4. Fixed fat
Twelve neutered adult female cats aged 2.4 to 4.5 years (mean ± s.e.m.=3.9±0.2 years) and weighing 4.86±0.20 kg participated in this experiment. This experiment had the same design as Expts 2 and 3, except that the three experimental foods had equal fat density but differed in their protein:carbohydrate ratio. Food compositions are shown by the black dots in Fig.4A.

Experiment 5. Paired fixed fat foods at different frequencies
Twelve neutered adult female cats aged 4.3 to 5.2 years (mean ± s.e.m.=4.7±0.1 years) and weighing 4.32±0.14 kg were used in this experiment, which built upon Expt 4 in that diets Pfc and pfC were simultaneously offered to cats but in differing ratios (relative number of bowls of each), namely 3:1, 2:2 and 1:3. This experiment had only two phases (the naive simultaneous self-selection phase was omitted). Food compositions are shown by the black dots in Fig.5A.

Phase 1: monadic diets, sequential self-selection
Cats were randomly assigned to a diet rotation and cycled through five 2 day periods in which they were confined to either diet Pfc or pfC on successive days. A single bowl of food was offered each day containing 150 g of the allocated diet, and was available to the cats from 10:30 to 08:30h the following morning.

Fig.2. Results from Expt 2, dry diets with fixed carbohydrate content. Symbols and conventions are as in Fig.1. Values in B–D are means ± s.e.m.; N=12 cats.
Fig. 3. Results from Expt 3, dry diets with fixed protein content. Symbols and conventions are as in Fig. 1. Values in B–D are means ± s.e.m.; N=11 cats.

Fig. 4. Results from Expt 4, dry diets with fixed fat content. Symbols and conventions are as in Fig. 1. Values in B–D are means ± s.e.m.; N=12 cats.
Phase 2: experienced simultaneous self-selection
This phase consisted of three blocks of 4 days during which each cat was given simultaneous access to four food dishes containing foods pfC and Pfc in frequencies of 3:1, 2:2 and 1:3 for four consecutive days. Cats were randomly assigned to three different groups to determine the order in which the differing frequencies of diets were offered. In addition, the order in which each bowl was positioned was changed daily within each 4 day block to eliminate possible bias associated with a favoured feeding position. Each bowl contained 150g of the allocated diets and was available to the cats from 10:30 to 08:30h the following morning.

Wet diet experiments
Only cats that had been fed on wet food from weaning were used in these experiments and no cat was used in more than one experiment.

Experiment 6. Fixed protein
Twelve neutered adult cats (seven female, five male) aged 4.5–11.1 years (mean ± s.e.m.=6.8±0.7 years) and weighing 4.98±0.34 kg were used. The design of this experiment was the same as Expt 3, but employed wet diets. Food compositions are shown by the black dots in Fig. 6A.

Experiment 7. Fixed fat
Twelve neutered adult cats (seven female, five male) aged 4.5–11.1 years (mean ± s.e.m.=6.6±0.7 years) and weighing 5.02±0.17 kg were used. The design of this experiment was the same as Expt 4, but using wet foods. Food compositions are shown by the black dots in Fig. 7A.

Experiment 8. Fixed carbohydrate
Twelve neutered adult cats (four female, eight male) were allocated to this experiment although one male was removed because of low food intake and loss of body mass; these data were excluded from analysis. Cats (N=11) were aged 3.6 to 12.7 years (mean ± s.e.m.=6.6±0.9 years) and weighed 5.14±0.27kg at the start of the experiment. The experimental design was the same as Expt 2, but using wet instead of dry foods. Food compositions are shown by the black dots in Fig. 8A.

Experiment 9. Variable protein, carbohydrate and fat
It was apparent from analysis of Expt 1 that cats were attempting to minimise carbohydrate intake but were unable to select less than 26% of their calorie intake from carbohydrate, as this was the lowest percentage available in the diets offered. We hypothesised that providing diets with lower carbohydrate content than those in Expt 1 would allow cats to demonstrate the target position for protein, fat and carbohydrate, free from the carbohydrate ceiling that constrained intake in the dry diet experiments. The design of the experiment was also modified to provide information on the ‘rule of compromise’ (sensu Raubenheimer and Simpson, 1997) in a no-choice situation. Thus, cats were restricted to the following regimes for 14 days: either confined to one of three single foods, which represent the apices of...
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Fig. 6. Results from Expt 6, wet diets with fixed protein content. Symbols and conventions are as in Fig. 1. Values in B–D are means ± s.e.m.; N=12 cats.

Fig. 7. Results from Expt 7, wet diets with fixed fat content. Symbols and conventions are as in Fig. 1. Values in B–D are means ± s.e.m.; N=12 cats.
Fig. 8. Results from Expt 8, wet diets with fixed carbohydrate content. Symbols and conventions are as in Fig. 1. Values in B–D are means ± s.e.m.; N=12 cats.

Fig. 9. Results from Expt 9, variable protein, carbohydrate and fat content. Black circles, daily intakes on fixed foods; green circles, intakes for self-selecting cats. Otherwise, conventions are as in Fig. 1. Values in B–D are means ± s.e.m.; N=12 cats for diet pFc and N=10 cats for all other diets.
the composition triangle in Expts 6–8 (Pfc, pFc, pFc), or provided all three simultaneously and allowed to self-select.

Initially, 48 neutered adult cats (16 female, 32 male) were housed and fed in four groups (four females and eight males per group). Three groups were fed a single diet (either Pfc, pFc or pFc) whilst the fourth group was offered all three diets simultaneously following four 3-day cycles of monadic feeding [i.e. confined to a different food (pFc, pFc or Pfc) on each of the three days]. All groups were fed in two 1 h periods per day (09:00–10:00h and 14:00–15:00h). During this group feeding period, six cats were removed from the study – four due to loss of body mass [three males and one female from diet groups Pfc (2), pFc (1) and self-selection (1)], one male due to recurrent poor faeces (self-selection group) and one male sustained a broken toe which required surgery to repair (diet pFc). Thus 42 cats aged 2.9 to 9.1 years (mean ± s.e.m. = 4.9±0.9 years) and weighing 4.95±0.15 kg went on to the individual feeding phase of this experiment. Cats were maintained on the same dietary regimen they had experienced during the group feeding period (i.e. single diet or simultaneous self-selection in two 1 h meals per day). At each meal, 175 g of each allocated diet was offered and any uneaten food at the end of each feeding period was weighed to allow calculation of the amount of each diet eaten. Food compositions are shown by the black dots in Fig. 9A.

**Statistical methods**

Two-tailed matched-pairs t-tests were used to compare the nutrient intakes of cats in different phases of the repeated-measures design experiments (Expts 1–8). A significance level of 5% was adopted for all tests. Tests were conducted using PASW Statistics v. 18 (SPSS, IBM, Somers, NY, USA).

**RESULTS**

**Dry diet experiments**

Experiment 1. Variable protein, carbohydrate and fat

The proportional compositions of diets self-selected by cats from all three phases clustered to the right and midway down the region of the accessible composition space (Fig. 1A). This shows that the cats regulated a macronutrient balance low in carbohydrate and with an intermediate protein:fat balance. The clustering of self-selected intakes can also be seen in intake space (Fig. 1B–D). Comparison of nutrient intakes further reveals that the experienced simultaneous self-selectors (phase 3) selected a diet with a higher protein:carbohydrate balance than the naive cats (phase 1) \( t_{110}=5.987, P<0.001 \) (Fig. 1B). A striking feature of these plots is that all carbohydrate intake points aligned tightly along a value of \( \sim 300 \)kJ d\(^{-1} \) (Fig. 1B,C). This suggests that \( \sim 300 \)kJ of carbohydrate is either a target level or a maximum tolerable level (ceiling) for the cats. The latter explanation is supported by data presented below.

**Experiment 2. Fixed carbohydrate**

The proportional compositions of diets self-selected by cats from all three phases clustered slightly to the high-protein side of the accessible composition space (Fig. 2A). This suggests that, when the food carbohydrate content is fixed at \( \sim 25\% \), the cats regulated a macronutrient intake roughly intermediate in protein:fat balance. Note the similarity in the point selected in this experiment and in Expt 1. Also similar to Expt 1 is the tight alignment of intake points on the carbohydrate axis of intake space (Fig. 2B,C). The fact that the cats reached this ceiling even on foods with fixed, relatively low (compared with food pFc) carbohydrate levels of ca. 25\% demonstrates that 25\% exceeds the carbohydrate target proportion for cats (see below). Unlike Expt 1, however, there was no marked difference between the self-selected points of the naive and experienced simultaneous self-selecting phases (P:C ratio, \( t_{1,10}=2.229, P=0.048 \); P:F, \( t_{1,10}=2.096, P=0.06 \)).

**Experiment 3. Fixed protein**

As in Expt 1, the intake points selected by naive and experienced simultaneous self-selectors differed (Fig. 3). In both cases, the experienced cats selected a lower proportion of carbohydrate in the diet than the naive cats (P:C ratio, \( t_{1,10}=12.234, P<0.001 \)). The sequential self-selectors compiled a diet intermediate between the diets of naive and experienced simultaneous self-selectors (blue circles). Plots of nutrient intakes again show evidence of a ceiling effect on carbohydrate intake (Fig. 3B,C), although unlike in Expts 1 and 2 in this case the cats on the pFc monadic diet treatment did not ingest carbohydrate to this level. Comparison of nutrient intakes by naive and experienced simultaneous self-selectors shows that the experienced cats achieved a higher protein intake than naive cats \( t_{1,10}=6.526, P<0.001 \), and in so doing also ingested a larger quantity of carbohydrate \( t_{1,10}=4.032, P=0.002 \) (Fig. 3B,C). As a consequence of the putative ceiling on carbohydrate intake, this increase in protein intake could only be achieved by avoiding the high-carbohydrate food – i.e. by increasing the proportions of the intermediate- and high-fat foods (pF and pFc, respectively) in the diet. In so doing, experienced cats also had increased intake of fat \( t_{1,10}=9.322, P<0.001 \) (Fig. 3C,D) and an increase in the fat:protein ratio of the diet \( t_{1,10}=5.610, P<0.001 \) (Fig. 3A,D) relative to naive simultaneous self-selecting and sequential self-selecting cats.

**Experiment 4. Fixed fat**

Naive and experienced simultaneous self-selecting cats mixed a diet with similar macronutrient balance (P:C ratio, \( t_{1,11}=1.937, P=0.079 \); F:C ratio, \( t_{1,11}=1.943, P=0.079 \) (Fig. 4A), and this balance was very close to the maximal P:C possible given the available diets. The diet selected by sequential self-selectors was similar, but had higher carbohydrate content (naive self-selectors vs sequential self-selectors, P:C ratio, \( t_{1,11}=5.672, P<0.001 \). Fig. 4B–D shows, as in previous experiments, that there was a distinct upper limit to carbohydrate intake. In this case, both the Pfc and pFc monadic diet treatments and the self-selected intake means also aligned tightly on a value for fat intake of \( \sim 240 \)kJ. A possible interpretation of this is that the cats encountered a daily fat intake limit of \( \sim 240 \)kJ. However, this cannot be the case, because Expts 1, 2 and 3 showed that cats are capable of eating considerably higher levels of fat, in excess of \( 600 \)kJ d\(^{-1} \). A second interpretation is that \( 240 \)kJ d\(^{-1} \) represents the target coordinate for daily fat intake. Results from Expt 8 with wet diets accord with this latter interpretation (see below).

**Experiment 5. Paired fixed fat foods at different frequencies**

Cats ate almost exclusively from the high-protein food (Pfc), even when it was present at a ratio of 1:3 dishes of high-carbohydrate food (pFc) (Fig. 5). As a result, the selected diet has almost the same proportional balance as the high-protein food. The results are in close agreement with those in Expt 4; hence the selection against the high-carbohydrate food was robust even with decreasing frequency of the high-protein food in the environment.

**Wet diet experiments**

**Experiment 6. Fixed protein**

Experienced simultaneous self-selectors and the sequential self-selectors compiled diets with very similar macronutrient balances (P:C ratio, \( t_{1,11}=0.214, P=0.835 \); F:C ratio, \( t_{1,11}=0.222, P=0.828 \) whereas the relative proportion of carbohydrate was higher in naive cats; \( t_{1,11}=2.229, P=0.048 \); P:F, \( t_{1,11}=2.096, P=0.06 \)).
self-selecting cats (naive self-selectors vs experienced self-selectors, P:C ratio; \( t_{1,11}=4.569, P<0.001 \); F:C ratio, \( t_{1,11}=2.222, P=0.082 \); Fig.6). Details of these comparisons can be seen in intake plots in Fig.6B,C. The nutrient intake plots further show that both simultaneous self-selection groups ingested appreciably more nutrients overall than did the sequential self-selection group (sequential self-selectors vs experienced self-selectors, \( t_{1,11}=15.698, P<0.001 \)). Together, these data suggest that an enforced diet switching interval of 1 day results in reduced nutrient intake, but has no impact on the balance of nutrients selected compared with experienced simultaneous self-selectors.

Experiment 7. Fixed fat
The diet composition of all self-selecting groups was similar (Fig.7A). As in Expt 6, the nutrient intake plots show that the balance of nutrients selected by the sequential and experienced self-selecting groups was similar (P:C ratio, \( t_{1,11}=1.909, P=0.083 \); F:C ratio, \( t_{1,11}=1.847, P=0.092 \), but the sequential self-selectors had lower nutrient intake overall than both groups of simultaneous self-selectors (sequential self-selectors vs experienced self-selectors, \( t_{1,11}=9.995, P<0.001 \)). Also as in Expt 6, the experienced simultaneous self-selectors compiled a diet with a lower proportion of carbohydrate than the naive group (naive self-selectors vs experienced self-selectors, P:C ratio, \( t_{1,11}=2.58, P=0.026 \)).

Experiment 8. Fixed carbohydrate
The proportional diet compositions of all self-selecting groups were closely similar (Fig.8A). As in Expts 6 and 7, the nutrient intake plots indicate that the balance of nutrients selected by the sequential and experienced self-selecting groups was similar (P:C ratio, \( t_{1,11}=2.09, P=0.063 \); P:F ratio, \( t_{1,11}=0.827, P=0.427 \) and the sequential self-selectors had lower nutrient intake overall than both groups of simultaneous self-selectors (sequential self-selectors vs experienced self-selectors, \( t_{1,11}=3.0075, P=0.013 \); sequential self-selectors vs naive self-selectors, \( t_{1,11}=11.72, P<0.001 \)).

Experiment 9. Variable protein, carbohydrate and fat
When offered a choice of the three foods, cats mixed them to achieve a daily intake of ca. 420kJ protein, 100kJ carbohydrate and 280kJ fat (Fig.9). When restricted to the high-carbohydrate food (pC), as in earlier experiments, cats did not exceed a carbohydrate intake of 200–300kJ and thus suffered a shortage in protein relative to the target of 420kJ day\(^{-1}\). Cats confined to the high-fat food (pF) similarly did not achieve the target intake for protein.

Energy intake and body mass changes
Table 3 presents a summary of total energy intakes across all experiments and highlights the marked variation in energy intake both within and between studies. The mean change in body mass of the cats amounted to only 3\% above or below their initial body mass over the duration of each experiment (except Expt 8, where a mean decrease in body mass of 5\% from the start was seen) (data not shown). Given the relatively small deviations in body mass of the cats over the duration of each experiment, energy intake would have been expected to be maintained at a relatively constant level across the different phases of each experiment if the diet selection patterns of the cats were based on regulation/maintenance of energy intake; this was clearly not the case. Of particular note is the lower intake of the pF diet during the monadic phase of Expts 1, 3, 4 and 5, reflecting the influence of carbohydrate in limiting total energy intake (carbohydrate ceiling effect), particularly when it is present at higher levels in the diet.

In Expt 9, where the diet choice regimen allowed the cats to achieve their macronutrient target intake, the actual energy intake (mean ± s.e.m.=819±45kJ day\(^{-1}\)) was somewhat lower than the predicted energy requirement (929kJ day\(^{-1}\)) based on an estimated maintenance energy requirement of 77.6kcal kg\(^{-0.71}\) (Bermingham et al., 2010). Furthermore, the actual energy intakes for the cats fed the high-protein, high-carbohydrate and high-fat diets (893±36, 725±31 and 665±48kJ day\(^{-1}\), respectively) were also lower than the predicted energy requirements (983, 1034 and 1030kJ day\(^{-1}\), respectively), reinforcing the view that energy intake was not the regulatory priority governing food intake in these cats.

DISCUSSION
This is the most extensive study of macronutrient regulation yet undertaken on any carnivore. We have been able to tease apart the complex interactions among protein, fat and carbohydrate using

| Experiment | Naive simultaneous self-selection | Monadic (sequential self-selection) | Experienced simultaneous self-selection |
|------------|----------------------------------|--------------------------------------|---------------------------------------|
| 1          | 819 (671–967)                    | pC                                   | pF                                   | 1001 (926–1076)                      |
| 2          | 823 (739–907)                    | pC                                   | Pfc                                  | 898 (816–980)                        |
| 3          | 669 (586–752)                    | pC                                   | Pfc                                  | 1211 (1036–1386)                     |
| 4          | 964 (881–1047)                   | pC                                   | Pfc                                  | 1057 (982–1132)                      |
| 5          | ND                               | pC                                   | ND                                   | 940 (866–1014)                       |
| 6          | 1418 (1298–1538)                 | pC                                   | pFc                                  | 1394 (1298–1490)                     |
| 7          | 1246 (1143–1349)                 | pC                                   | pFc                                  | 1226 (1121–1331)                     |
| 8          | 1369 (1258–1480)                 | pC                                   | Pfc                                  | 1116 (992–1240)                      |
| 9          | ND                               | pC                                   | Pfc                                  | 819 (731–907)                        |

Mean energy intakes are shown with 95% confidence intervals in parentheses. The details of diets used in experiments 1–5 and 6–9 are shown in Tables 1 and 2, respectively. ND, not determined.
geometric techniques that combine mixture triangles (Emmans, 1991) and intake plots from the geometric framework (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993). The geometric framework allows two important parameters of nutritional regulation to be defined and measured: the intake target and rules of compromise. The intake target is the amount and balance of nutrients that supports optimal performance (in Darwinian fitness terms) and to which regulatory systems might be expected to aim (Simpson et al., 2004). Rules of compromise define the relative weighting given to regulation of different nutrients when animals are confined to imbalanced diets (Simpson and Raubenheimer, 1995). We consider each of these in turn, and then discuss conclusions that may be drawn about the behavioural mechanisms involved.

**Intake target position**

When all experiments are superimposed upon a single composition triangle (Fig. 10), it can be seen that cats mixed diet compositions that approached as closely as possible the composition achieved by self-selecting cats in Expt 9 (red star, Fig. 10). Note, for example, in Fig. 10 how, except for Expt 3 in which animals were confined to the basal edge of the dry diet region (orange triangle), the cats in the dry diet experiments all converged to the apex of the triangle, close to the wet diet region (blue triangle). Similarly, cats in the wet diet treatments from Expts 6–8 arrived at the nearest point to the red star along their respective sides of the blue triangle. Maintenance of energy intake alone cannot explain this pattern, as energy intakes varied markedly within and between experiments (Table 3). Rather, the weight of evidence suggests that cats regulated the macronutrient composition of the diet towards a target composition lying within the region bounded by the wet diets. We estimate from the data in Expt 9 that the intake target lies close to 26 g day\(^{-1}\) protein, 9 g day\(^{-1}\) fat and 8 g day\(^{-1}\) carbohydrate, yielding a macronutrient energy composition of 52% protein, 36% fat and 12% carbohydrate.

As an obligate carnivore, the cat has certain metabolic adaptations that result in nutritional requirements (e.g. for arginine, taurine, vitamin A, vitamin D and arachidonic acid) being met adequately only through a diet based on animal tissue (MacDonald et al., 1984; Zoran, 2002). A macronutrient profile that is high in protein and fat is consistent with an evolutionary adaptation to a meat-based diet. These intake target ratios are similar to those reported for predatory fish (55% protein by energy) (Sanchez-Vazquez et al., 1999; Rubio et al., 2003; Ruohonnen et al., 2007) and somewhat higher for protein than that found by Mayntz et al. for mink (35% protein) (Mayntz et al., 2009).

**Rules of compromise**

Expt 9 allows the rule of compromise to be determined. Results from this experiment showed that, in a sustained no-choice situation, both excess fat and carbohydrate imposed constraints on protein gain. Results from the other experiments indicated that this is an absolute constraint for carbohydrate (see below), whereas under circumstances where protein was very low (e.g. see fat intake on pF in Fig. 3D), the limit on fat intake was more flexible. When confined to a high-protein diet in Expt 9, cats overshot protein intake relative to the target, presumably to gain energy (a limiting resource). The extra gain in fat and carbohydrate thus achieved was small, however, suggesting that the major source of energy came from protein itself. An ability to overingest protein relative to the intake target has also been reported in predatory beetles (Raubenheimer et al., 2007), mink (Mayntz et al., 2009) and European whitefish (Ruohonnen et al., 2007). These predators contrast with the herbivores and omnivores studied to date, which maintained more stable absolute protein intake in the face of dietary imbalance (e.g. Simpson and Raubenheimer, 2005; Felton et al., 2009). That animals at higher trophic levels should have to obtain an increasing proportion of their energy from protein is to be expected from theory [see fig. 4 in Raubenheimer et al. (Raubenheimer et al., 2009)].

**The carbohydrate ceiling**

It is clear, from the dry diet experiments particularly, that there was a carbohydrate intake ceiling for cats at ca. 300 kJ day\(^{-1}\) (e.g. see the horizontal alignment at this value in Fig. 1B,C). This is seen particularly in the dry diet Expts 1, 3, 4 and 5 in which diet pF was used. Protein intake on days during phase 2 when cats were fed diet pF was 134, 102, 132 and 141 kJ day\(^{-1}\) compared with the target of 420 kJ day\(^{-1}\). Furthermore, energy intake on days that cats were fed diet pF during phase 2 of these studies was 514, 475, 552 and 559 kJ day\(^{-1}\) compared with ~1000 kJ day\(^{-1}\) on the days the other diets were used (see Table 3). Based on a maintenance energy requirement for adult cats of 77.6 kcal kg\(^{-1}\) day\(^{-1}\) (Birmingham et al., 2010), which equates to ~1017 kJ day\(^{-1}\) for a 5 kg cat, it can be seen that consumption of dry diet pF resulted in deficits of both energy and protein intake (relative to the target). The carbohydrate ceiling explains many of the intake patterns seen in both dry and wet diet experiments and suggests that cats may only be able to process ingested carbohydrate up to a certain level. Cats have a number of sensory and metabolic adaptations that reflect their expected low carbohydrate intake (Eisert, 2011), including the absence of a functional sweet taste receptor (Li et al., 2005), low rates of intestinal glucose uptake (Buddington et al., 1991), a lack of salivary amylase and reduced activities (compared with dogs, for example) of pancreatic amylase and intestinal disaccharidases (Meyer and Kienzle, 1991). The reduced enzymatic capacity for digesting carbohydrate may mean that high carbohydrate intake could have untoward effects on cats, with any carbohydrate escaping digestion.
in the small intestine passing to the colon and providing substrate for microbial fermentation. Indeed, high carbohydrate intake in cats has been shown to increase the concentration of organic acids (i.e. end-products of microbial carbohydrate metabolism) in the colon and faeces and reduce faecal pH, indicating acidosis of the large bowel (Meyer and Kienzle, 1991).

Studies on salmonid fish have similarly reported a threshold level for carbohydrate intake beyond which food intake is reduced (Ruohonen et al., 2007) and performance falls precipitously (Vielma et al., 2003). This contrasts with omnivores and herbivores, many of which have been shown to metabolise excess ingested carbohydrate (Zanotto et al., 1997; Stock, 1999; Trier and Mattson, 2003). Such a mechanism both ameliorates excess fat storage and allows elevated food consumption to gain limiting nutrients, notably protein (Simpson and Raubenheimer, 2005).

Understanding of carbohydrate metabolism in the cat is incomplete but there is evidence to suggest that the ability of cats to metabolise excess ingested carbohydrate is limited. The glycogen storage capacity of cats is not known, but in humans it is limited to ~15 g kg⁻¹ body mass; once this is saturated, further glucose is disposed of by increasing carbohydrate oxidation and de novo lipogenesis (Acheson et al., 1988). In cats, de novo lipogenesis is not thought to be an important pathway for glucose metabolism. In vitro work showed that acetate is favoured over glucose as a carbon source for de novo fatty acid synthesis in feline adipocytes and, in particular, glucose was not converted into fatty acids in feline hepatocytes (Richard et al., 1989). Whilst increasing carbohydrate oxidation may, in theory, represent a means of disposing of excess glucose in cats, the key step in this process is the cellular uptake of glucose. This may be limited in cats because feline tissues do not express the high-capacity glucose-phosphorylating enzyme glucokinase (Ballard, 1965; Tanaka et al., 2005), which is required to remove intracellular glucose in order to maintain a concentration gradient for further glucose uptake. Instead cats rely on the high-affinity but low-capacity hexokinase, which may limit the ability of cats to rapidly oxidise a large amount of ingested carbohydrate. A priority for future research should be to establish whether the carbohydrate constraint in cats is mediated at the level of the alimentary canal or post-absorptively.

Effect of experience

Experienced self-selecting cats on dry diets ate a lower proportion of carbohydrate relative to protein compared with naive self-selecting cats, mainly by increasing protein intake (note how the red circles in Fig. 1B to Fig. 4B lie further along the protein intake axis than the green circles). The shift with experience on dry diets is therefore consistent with learning to avoid exceeding the carbohydrate ceiling. These results provide yet another example of a consistency in mechanisms across guilds of consumers: learning has been demonstrated to play a role in macronutrient balancing in omnivorous and herbivorous insects (Simpson and White, 1990; Raubenheimer and Tucker, 1997; Gadd and Raubenheimer, 2000), predatory fish (Rubio et al., 2003) and rodents (Baker et al., 1987).

Effect of enforced switching intervals (sequential self-selection)

When forced to switch between dry diets over 1 day periods, cats were unable to avoid the carbohydrate ceiling and hence underate protein relative to experienced simultaneous self-selectors [note how the blue circles are to the left of and on a steeper nutritional rail (lower P:C ratio) than the red circles in Fig. 1B to Fig. 4B]. In contrast, on wet diets the P:C ratios ingested were similar (i.e. lay on the same nutritional rail) between sequential self-selectors and simultaneous self-selectors; however, the total amounts ingested by sequential self-selectors were invariably lower (Fig. 6B to Fig. 8B). These results suggest that, although not as constrained by carbohydrate as in the dry diet treatments, there were constraints on total intake imposed by 24 h restriction to single wet foods that were not evident in cats able to switch between diets ad libitum. An inability to maintain long-term intake when confined for extended periods on single imbalanced diets has been associated with a specialist as compared to a generalist feeding strategy (Raubenheimer and Jones, 2006).

A cautionary tale

Ours is the second published study of which we are aware that explores macronutrient regulation in domestic cats. The previous study by Cook et al. (Cook et al., 1985) claimed not to find regulation of protein intake in kittens. However, if their published data are recast using our geometric protocol, a very different story emerges. Kittens did, in fact, show impressive regulation of protein intake. For example, calculations based on the food intakes provided in fig. 1 in Cook et al. (Cook et al., 1985) show that kittens in all three diet pairings consumed close to 25 g of protein per day, despite the food pairings being widely different [18% protein (in energy terms) vs 38%, 18 vs 54%, or 36 vs 54%]. To do so, kittens overate non-protein energy, resulting in a daily total energy intake for the lower-protein pairings that was twice the value of the higher protein pairings.

This illustration reinforces the need to employ geometric analysis in the design and interpretation of multivariate nutritional experiments. We have considered all three macronutrients in the present study. An important issue for future research is the extent to which other dietary qualities such as the texture, water content and additional flavours impact the regulation of these nutrients.

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