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Obligate herbivory in an ancestrally carnivorous lineage: the giant panda and bamboo from the perspective of nutritional geometry

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Summary

1. Herbivores face various nutritional challenges in their life cycles, challenges that may become increasingly acute under ongoing environmental changes.
2. Here, focusing on calcium, phosphorus and nitrogen, we used nutritional geometry to analyse individual-based data on foraging and extraction efficiencies, and combined these with data on reproduction and migratory behaviour to understand how a large herbivorous carnivore can complete its life cycle on a narrow and seemingly low quality bamboo diet.
3. Behavioural results showed that pandas during the year switched between four main food categories involving the leaves and shoots of two bamboo species available. Nutritional analysis suggests that these diet shifts are related to the concentrations and balances of calcium, phosphorus and nitrogen. Notably, successive shifts in range use and food type corresponded with a transition to higher concentrations and/or a more balanced intake of these multiple key constituents.
4. Our study suggests that pandas obligatorily synchronize their seasonal migration and reproduction with the disjunct nutritional phenologies of two bamboo species. This finding has potentially important implications for habitat conservation for this species and, more generally, draws attention to the need for understanding the nutritional basis of food selection in devising management plans for endangered species.

Key-words: feeding strategy, giant panda, life cycle, nutritional geometry, reproductive timing, right-angled mixture triangles, seasonal migration

Introduction

It is widely accepted that herbivores face nutritional challenges, including foods that vary in poorly digestible fibre (Milton 1979), plant-produced toxins (Rosenthal & Berenbaum 1991) and nutritionally imbalanced foods (Elser *et al.* 2000, 2007; Ritchie 2000). Such nutritional challenges may grow worse under ongoing climate change that shifts the range, timing and physiological conditions of forage plants (Tuanmu *et al.* 2012). In response to such challenges, animals have evolved behavioural, developmental and physiological adaptations that interact across time-scales to facilitate homeostasis and maintain performance (Mayntz *et al.* 2005; Rothman *et al.* 2011). The science of nutritional

ecology aims to understand the ways that these adaptations mediate the relationships between nutrient needs and ecological constraints (Raubenheimer, Simpson & Mayntz 2009). This helps to inform our understanding of the ecological and evolutionary processes that have shaped the diversity of animal foraging modes and to devise management strategies for endangered species and their habitats (Moore & Foley 2005; Raubenheimer, Simpson & Tait 2012).

An important response to variation in nutrient supply or demand (e.g. at different stages in the life cycle) is compensatory adjustment in foraging behaviour and physiological processing of nutrients (Raubenheimer, Simpson & Tait 2012). Foraging adjustments typically involve behaviours that balance the gain of several nutrients (e.g. usable energy, protein and amino acids, minerals), and physiological

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adjustments can involve changes in allocation to reproduction or other traits with particular nutritional demands. Animals that live in seasonal environments may be especially likely to adjust foraging and physiology to meet diverse nutritional challenges (Goldizen *et al.* 1988; Rubenstein & Wikelski 2003).

The giant panda (*Ailuropoda melanoleuca*) is an endangered, obligate herbivore that diverged early within an otherwise carnivorous clade (Qiu & Qi 1989; Wei *et al.* 2012; Zhao *et al.* 2013). Uniquely within the order Carnivora, pandas specialize (*c.* 99%) on various species of bamboo, resulting in a diet that is generally believed to be of poor quality due to low protein and high fibre and lignin contents, contributing to its low dry matter digestibility (Schaller *et al.* 1985; Hu *et al.* 1990; Wei *et al.* 1999; Zhu *et al.* 2011). Despite being exclusively herbivorous, the giant panda retains the simple stomach and short gastrointestinal tract typical of carnivores (Dierenfeld *et al.* 1982), and consequently needs to eat large amounts of poorly digestible foods (Hu *et al.* 1990). This high degree of specialization on large quantities of low quality food taken from a small number of plant species renders the giant panda vulnerable to extinction in the face of environmental change (Colles, Liow & Prinzing 2009). Further, its recently acquired herbivorous lifestyle provides an especially interesting opportunity for evaluating how shifts in herbivore behaviour allow a species to cope with food quality challenges involving the levels and balance of essential nutrients.

Previous studies have highlighted a number of interesting ecological and life-history characteristics that might be associated with the ability of panda populations to survive as bamboo specialists (Schaller *et al.* 1985; Pan *et al.* 2001). Nevertheless, these relationships remain poorly understood, largely because of the difficulty of obtaining intensive behavioural data for these secretive animals. Other bears give birth to unusually small, altricial young, a trait that has been associated with hibernation (Garshelis 2004). However, pandas do not hibernate and yet they have the shortest gestation period (3–5.5 months) and give birth to offspring that are the smallest of any bear species (Garshelis 2004; merely 0.1% of the mother's weight). As with other bears, pandas have an embryonic diapause, known as seasonal delayed implantation, in which the embryo remains suspended in the uterus in a state of arrested development until it attaches and resumes growth, sometimes months later (Schaller *et al.* 1985). While the adaptive significance of this remains uncertain (Thom, Johnson & MacDonald 2004), delayed implantation is believed to be an ecological adaptation to adjust the timing of mating and the rearing of offspring to different seasonal environments (Sandell 1990).

In this study, we used a combination of direct behavioural observations and individual-based characterization of food intake and egestion to assess the nutritional consequences of the seasonal food choices of pandas. We relate these choices to the timing of altitudinal migration and other major life-history events including seasonal mortality,

mating, gestation, parturition and lactation. We focused our analysis on the mineral nutrients calcium and phosphorus, as well as nitrogen as a proxy for protein, because of the critical roles that these nutrients play in growth and reproduction of animals, including mammals (White 1993; McDowell 1996; Moen, Pastor & Cohen 1999; Sterner & Elser 2002). Importantly, mammalian requirements for calcium and phosphorus, principally for bone growth, are critically interdependent (Van Soest 1994; Underwood & Suttie 1999) and thus of special interest in the study of nutritional ecology of vertebrate herbivores. To examine the roles of these multiple dimensions of nutritional quality and their interdependencies, we organize our analysis using nutritional geometry, an approach for modelling the interactive effects of nutrients on animals (Raubenheimer 2011). Our objectives were i. to elucidate the relationships between the distinctive adaptations of these newly obligate herbivores, seasonal habitat choice, food selection and nutrient gain in the extreme nutritional environment to which they have become specialized and ii. to learn whether the respective foods and habitats of the giant pandas are interchangeable (alternative sources of the same resources), or complementary (provide different combinations of essential nutrients). The study thus provides fundamental insight into the nutritional ecology of a highly unusual and ecologically threatened herbivore, as well as critical information for the management and conservation of panda habitat.

Materials and methods

STUDY SITE AND ANIMALS

This study was conducted in Foping Reserve, a key panda reserve, in the Qinling Mountains, China. The Qinling Mountains contain a high density of wild giant pandas with a population of 273 individuals (State Forestry Administration, China 2006). Two bamboo species are the main diet resource of the pandas there, wood bamboo (*Bashania fargesii*) and arrow bamboo (*Fargesia qinlingensis*), which grow at mean elevations of 1600 and 2400 m, respectively. These two bamboo species have different life histories. Wood bamboo (*WB*) produces shoots in May, and the shoots begin to sprout abundant new leaves in August. In contrast, arrow bamboo (*AB*) produces shoots in early June and its shoots sprout a limited number of new leaves in the following spring and considerably more new leaves in summer. The leaves of *WB* persist year-round, while the *AB* leaves drop off in winter.

With approval from the State Forestry Administration in China (2009-261), a total of six pandas, three adult females and males, respectively, were fitted with GPS/VHF collars (Lotek Wireless Inc., Ontario, Canada; Nie *et al.* 2012a,b; Zhang *et al.* 2014). This made it possible to conduct intensive behavioural observations and collect food samples, enabling us to determine individual-level seasonal food intake and obtain paired food–faecal samples for chemical analysis and assessment of relative digestive extraction efficiencies.

OBSERVATIONS OF FORAGING BEHAVIOUR AND SAMPLE COLLECTION

Using the GPS collars, over 6 years, we tracked pandas from short distances (usually 10–20 m) to examine their seasonal pattern of

food selection. Food and dung samples were collected in the four foraging seasons of 2009 and 2010. During this period, we also tracked pandas to conduct behavioural observations at intervals of 3–5 days for each individual, except when inclement weather prevented this. These observations enabled us to determine which bamboo species, tissues and ages were chosen by pandas. Paired food and fresh faeces samples were collected at each observed feeding patch during different foraging periods year-round. We defined a feeding patch as an area with a size of *c.* 300 × 300 m within which a panda was observed feeding for at least 24 h (because the gut passage time is usually around 10–12 h; Schaller *et al.* 1985). Bamboo leaf and shoot samples were collected according to the age of plants; that is, one- and multiyear old leaves, and new and old shoots, respectively. All food and faecal samples were coded by the feeding patch, dried in the field station, and the plant samples were sorted by different bamboo species and tissues. The dried plant and faecal samples were stored in zip-lock bags in the field for transport to laboratory.

LIFE CYCLE AND MORTALITY DATA COLLECTION

To examine the possible relationship between nutrition and reproduction strategy, we conducted a study of the reproductive ecology of giant pandas by tracking collared animals over 6 years from 2007 to 2012 (Nie *et al.* 2012a,b). We also analysed long-term (37 years) historical data of panda death and illness events in the wild from Foping Reserve records.

These data were used to explore the potential effect of food resource quality on the individual life span and population dynamics of this endangered species.

LABORATORY ANALYSES

A total of 263 plant and faecal samples were collected in the field, including 66 shoots and 47 faecal samples during shoot foraging season, and 100 leaf and 50 faecal samples in leaf foraging season. All samples were ground to powder with a common multifunctional laboratory mill and oven-dried at 70 °C and then weighed before laboratory analyses. We used the micro-Kjeldahl method (Bremner 1996) to analyse N concentrations (% of dry mass). P contents (% of dry mass) were measured by the ammonium molybdate method after persulphate oxidation (Kuo 1996), standardized against known reference materials. Ca contents (% of dry mass) were determined using an atomic absorption spectrometer after hydrofluoric acid oxidation (Langmyhr & Thomassen 1973).

DATA ANALYSIS

We used Right-angled Mixture Triangles (RMTs, Raubenheimer 2011) to explore the relationships among the proportional contents of nutrients in the foods and published estimates of nutrient requirements. To estimate digestive extraction efficiencies of Ca, P and N, we compared the proportional compositions of food samples with the associated faeces using RMTs. This method does not yield the absolute digestive efficiencies of separate nutrients, for which measures are needed of the absolute intake and excretion of each nutrient. Rather, by comparing the concentrations of nutrients in the food and matched faeces, we are able to establish the *relative* extraction efficiencies of the focal nutrients (Raubenheimer 2011). For example, if the concentration of P in the faeces was half that in the matched foods, we could not conclude that P was extracted with 50% efficiency, because we would not know the extent to which the change in P concentration from food to faeces was due to the extraction of other nutrients (i.e. changes in the denominator rather than numerator in the concentration ratio).

However, if the Ca : P ratio in the food was twice the Ca : P ratio in the faeces, then, we could conclude that Ca was extracted with higher efficiency (by a factor of 2) than P. Since in this analysis we were interested in the relative extraction efficiencies of Ca, P and N, we chose to use an RMT model in which each nutrient was expressed as a percentage of the sum of the three nutrients [e.g. % Ca = Ca/(Ca + P + N) × 100] rather than as a percentage of the total sample mass (i.e. grams Ca/100 g sample). This enables the relative digestive efficiencies of all three nutrients to be compared in a single model, and also excludes from the denominator unaccounted components that might otherwise confound the comparison of relative extraction efficiencies (Raubenheimer 2011). We could then use as a baseline the null model in which all three nutrients are extracted from the food with equal efficiency, indicated in RMTs as the situation where the Ca–P–N ratio of faeces and the associated food is the same (i.e. the composition points for food and faeces are superimposed). Alternative outcomes would be indicated by the vectors of displacement of faeces composition relative to food; for example, if the Ca : P ratio in faeces is lower than in food, this indicates that Ca was extracted with higher efficiency than P.

We used *t*-tests to compare the concentrations and ratios of nutrients in the foods of pandas and to compare the ratios of nutrients in the foods with matched faecal samples to establish relative digestive efficiencies (as explained above). Levene's Test was used to test for equality of variances, and where the null hypothesis of equal variances was rejected, we applied a modified *t*-test that does not assume equal variances. A Kolmogorov–Smirnov Test was used to compare the observed monthly frequencies of mortality with the random null model. One-sample *t*-tests were used to compare dietary calcium: phosphorus ratios with the required ratios from the literature. All tests were performed using IBM SPSS v. 20 (IBM Corp., Armonk, NY, USA).

Results

SEASONAL MIGRATION, FEEDING, REPRODUCTION AND MORTALITY

Seasonal movement and foraging patterns

A mean of 52.5 ± 6.5 observation days were collected year-round for each animal with a range of 43–61 days. Over an annual cycle, pandas fed on two bamboo species located at different elevations, which we refer to as winter habitat and summer habitat (Figs 1 and 2). All six collared pandas in this study showed a similar pattern of foraging transition, switching to *WB* shoots in early May (range: 30 April–4 May) when the shoots reached a height (31.0 ± 5.75 cm) sufficient that pandas could eat them. In early June (3 June–11 June), pandas moved to higher elevation and switched to *AB* shoots, within a short period from 3 June to 11 June at the time that low-elevation *WB* shoots had grown tall (247 ± 23.6 cm) and become lignified. Similarly, pandas switched to *AB* leaves when the shoots of *AB* had grown tall (233 ± 12.1 cm). The mean time of the migrations to low elevation was at the end of August, within the period of 12 August–10 September. Finally, pandas preferred the younger leaves during the two leaf periods. Thus, based on the seasonal migration and the specific tissues eaten from these two bamboo species, there were four distinct foraging periods, two in which leaves

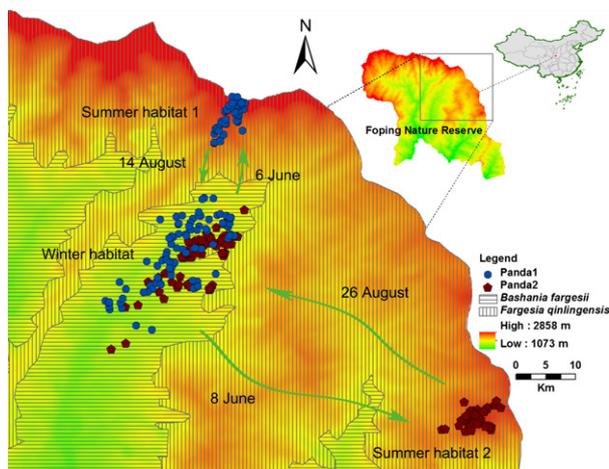


Fig. 1. Annual pattern of migration of giant pandas corresponding with a diet transition between two bamboo species in our study area. We used the GPS location data of two of the GPS collared pandas to exemplify the seasonal foraging migration pattern of giant pandas in this area. All the six collared pandas in our study lived in the same winter (September–May) habitat area at low elevation where wood bamboo (*Bashania fargesii*) is located. In summer, two of them moved to summer habitat 1 while four moved to summer habitat 2, at high elevation where arrow bamboo (*Fargesia qinlingensis*) is located.

and two in which shoots were eaten: wood bamboo leaf period (*WBL*), wood bamboo shoot period (*WBS*), arrow bamboo shoot period (*ABS*) and arrow bamboo leaf period (*ABL*, Fig. 2). During the *ABL* period, pandas sometimes ingested a small portion of the stems when eating leaves, but we did not include these infrequent occurrences in the data analysis.

Reproductive timing in giant pandas

Over several consecutive years, March (15 mating events; 68%) and April (seven mating events; 32%) were the main months for mating for the Qinling Mountain pandas. Pregnancy lasted 4–5 months (collared females: 137–143 days;

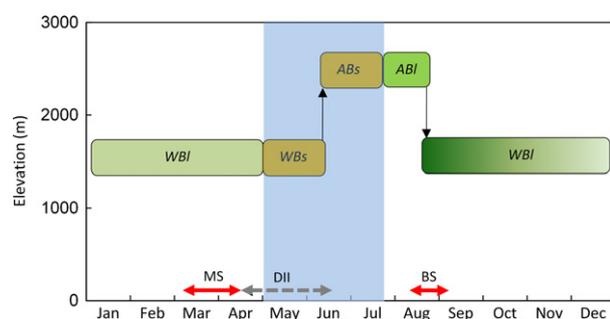


Fig. 2. Scheme relating the annual pattern of diet selection and habitat elevation to the reproductive cycle of giant pandas. Foods: *WBL*, wood bamboo leaves; *WBS*, wood bamboo shoots; *ABS*, arrow bamboo shoots; *ABL*, arrow bamboo leaves. Life-history events: MS, mating season, DII, delayed implantation interval, BS, birthing season.

146–151 days; 132–138 days), of which 1.5–2 months comprised postimplantation gestation (Schaller *et al.* 1985). The females gave birth in a short period between mid-August and early September: there were four births in mid-August (57%); two births in late August (29%) and one in early September (14%) during our study (Fig. 2).

Seasonal pattern of mortality of giant pandas

A total of 25 dead or ill pandas were observed in the wild over the past 37 years in Foping Reserve. More than half (52%) of these occurrences were in March and April, a frequency that was statistically greater than expected by chance ($Z = 1.61$; $P = 0.01$). This period corresponds to the end of the longest time on any of the four diets – the *WB* leaf period (Fig. 2).

NUTRITIONAL COMPOSITION OF FOODS

Nitrogen–phosphorus relationships

Figure 3a shows the composition of the seasonal diets in terms of N and P and a range of N to P ratios that are likely to encompass biomass requirements of mammals based on proximate chemical composition and investment in muscle and bone for an animal of this body size (Elser *et al.* 1996). The nutritional implications in relation to N and P for pandas for the spring switch from old *WB* leaves to *WB* shoots are denoted by the solid arrow labelled 1 in Fig. 3a. Young shoots (early May) had substantially higher concentrations of N ($P < 0.001$) and P ($P < 0.001$) than the leaves, with disproportionately more P and hence a lower N : P ratio ($P < 0.001$). As the shoots matured through May and early June, the concentrations of both N ($P < 0.0001$) and P ($P < 0.0001$) dropped, but the N : P ratio remained unchanged ($P = 0.097$).

The arrow marked 2 in Fig. 3a shows nutritional changes associated with the switch from old *WB* shoots to young *AB* shoots in June (see Fig. 2). The N : P ratio did not differ between the foods ($P = 0.98$), but the concentrations of both N ($P < 0.0001$) and P ($P < 0.0001$) were higher in *AB* shoots, indicating a shift to foods that allow greater intake of these nutrients.

By mid-July the concentrations of both N and P in the *AB* shoots had significantly declined ($P < 0.001$), although the N : P ratio barely changed ($P = 0.057$). This corresponded with a switch in July from the older shoots to young leaves of *AB* (solid arrow marked 3 in Fig. 3a), which were higher both in N ($P < 0.0001$) and P ($P < 0.0001$), with an increased N : P ratio ($P < 0.0001$).

Between mid-July and August, the concentrations of N ($P < 0.0001$) and P ($P < 0.0001$) had decreased in *AB* leaves, and the N : P ratio had increased from 11.9 ± 0.35 to 13.9 ± 0.57 ($P = 0.005$), whereupon female pandas migrated to the lower altitude foraging site and fed on young leaves of *WB*. These leaves had a significantly higher P content ($P = 0.016$) with similar N content

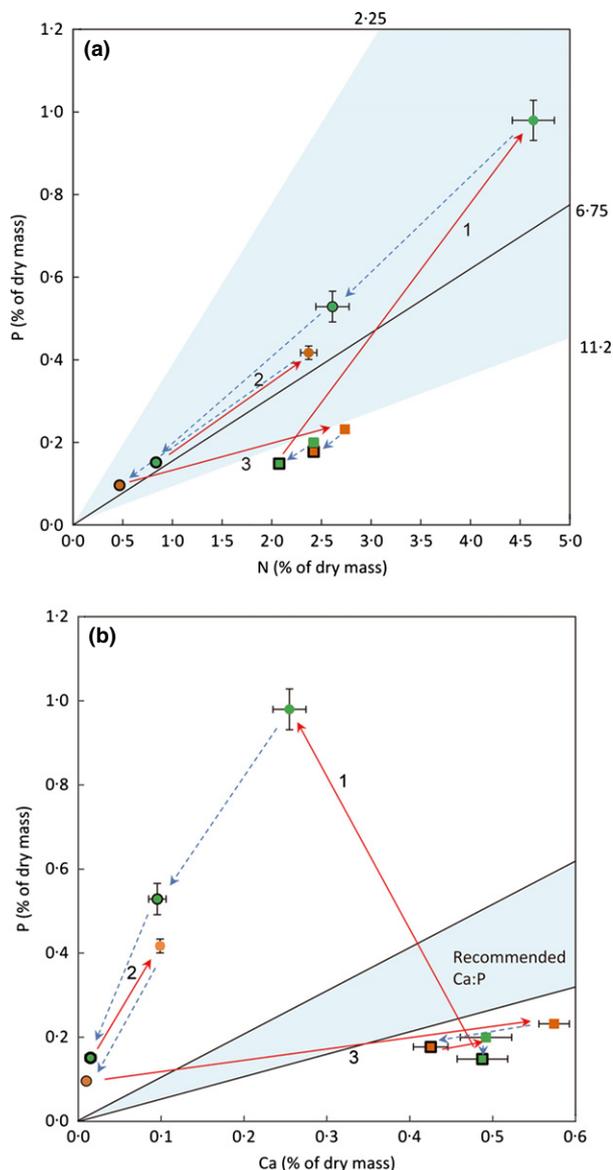


Fig. 3. Implications in terms of N:P (a), Ca : P (b) of seasonal diet shifts in giant pandas. Green = wood bamboo (*WB*), orange = arrow bamboo (*AB*); circles = shoots, squares = leaves; no border on symbols = young tissue; thin border = intermediate, thick border = old. Solid red arrows show active switches by pandas between foods, dashed blue arrows = seasonal changes in composition of foods being eaten. The shaded area denotes the recommended range for N–P, Ca–P ratios in the diets of mammals.

($P = 0.997$), and consequently a lower N : P ratio ($P = 0.012$) than the older *AB* leaves.

From August to April, when the pandas once again switched to young shoots of *WB* (arrow 1 in Fig. 3a), the concentrations of both N ($P < 0.0001$) and P ($P < 0.0001$) in *WB* leaves decreased, and the N : P ratio increased ($P = 0.002$).

Calcium–phosphorus relationships

The relationships between Ca and P in the dietary transitions by pandas through the annual cycle are shown in

Fig. 3b. The significant increase in dietary P concentration (see above) corresponding with the spring switch from leaves to shoots of *WB* (Fig. 2) was accompanied by a reduction in Ca ($P < 0.0001$), and consequently a strong decrease in the Ca : P ratio ($P < 0.0001$). Thereafter, as the shoots aged, the concentration of Ca dropped ($P < 0.0001$) together with the concentration of P (see above). However, Ca dropped more steeply than P, resulting in a significant reduction in the Ca : P ratio ($P < 0.0001$). The Ca : P ratios of shoots in the early, mid and late season were 0.26 ± 0.02 , 0.19 ± 0.02 and 0.09 ± 0.03 , respectively; all of these are substantially lower than the Ca : P ratio of 1–2 recommended in the diets of mammals (Van Soest 1994; Underwood & Suttie 1999; Buchman & Moukarzel 2000) ($P < 0.0001$). In contrast, the mean Ca : P ratio of mature *WB* leaves was 3.4 ± 0.24 , which is significantly higher than the maximum of two recommended for mammals ($P < 0.0001$).

The switch from the old shoots of *WB* to young shoots of *AB* (arrow 2 in Fig. 3b) was associated not only with a significant increase in P (see above), but also with a proportionately larger increase in Ca ($P < 0.0001$). Consequently, the Ca : P ratio was higher (closer to the recommended range) in young *AB* (0.25 ± 0.02) than the older *WB* shoots (0.09 ± 0.03 , $P < 0.0001$). As *AB* shoots aged, there was a reduction in P (see above) and Ca ($P < 0.0001$), and a decrease in the Ca : P ratio from 0.25 to 0.10 ($P < 0.0001$).

In mid-July, when the pandas switched from shoots to young leaves of *AB* (arrow 3 in Fig. 3b), the leaves were significantly higher in both P (above) and Ca ($P < 0.0001$), with a substantially higher Ca : P ratio ($P < 0.0001$). At a value of 0.10 ± 0.02 , the Ca : P ratio of shoots was an order of magnitude lower than the recommended minimum for mammals of 1 ($P < 0.0001$), while the value for leaves (2.49 ± 0.08) was marginally but significantly greater than the recommended maximum ratio of 2 ($P < 0.0001$).

By mid-August, the leaves of *AB* had reduced in both P (see above) and Ca ($P < 0.0001$) concentrations, but the Ca : P ratio remained unchanged ($P = 0.84$). At this point, the female pandas moved to a lower elevation and switched to the younger leaves of *WB*, which had a significantly higher P content (above) and marginally higher Ca ($0.51 \pm 0.04\%$ vs. $0.43 \pm 0.02\%$; $P = 0.08$). The Ca : P ratio did not differ between the leaves of the two species ($P = 0.97$).

From mid-August, when female pandas started eating young *WB* leaves, to April when they switched from the new older leaves to young *WB* shoots (arrow 1 in Fig. 3b), P concentration in the leaves dropped (above) but there was no change in Ca ($P = 0.63$). Consequently, the dietary Ca : P ratio of the *WB* leaves increased from 2.5 ± 0.13 in mid-August to 3.4 ± 0.24 in April ($P = 0.002$).

Relative digestive extraction efficiencies

Our analysis (Table 1) showed that, relative to N, both Ca and P were enriched in faeces compared with shoots,

Table 1. Comparison of the N, P and Ca concentrations and Ca/P ratio in foods (shoots and leaves) from two species of bamboo and the faeces produced by giant pandas from eating the respective foods

Component	<i>Bashania fargesii</i> (wood bamboo)		<i>Fargesia qinlingensis</i> (arrow bamboo)	
	Leaves	Shoots	Leaves	Shoots
N	$t_{(38-270)} = 8.150, P < 0.0001$	$t_{(30-016)} = 11.731, P < 0.0001$	$t_{(58)} = 4.197, P < 0.0001$	$t_{(23-083)} = 15.464, P < 0.0001$
P	$t_{(38-810)} = 1.375, P = 0.177$	$t_{(37-258)} = -9.095, P < 0.0001$	$t_{(58)} = 0.640, P = 0.524$	$t_{(23-782)} = -10.914, P < 0.0001$
Ca	$t_{(39-741)} = -8.791, P < 0.0001$	$t_{(26-320)} = -9.974, P < 0.0001$	$t_{(58)} = -4.141, P < 0.0001$	$t_{(22-970)} = -11.942, P < 0.0001$
Ca/P	$t_{(36-798)} = -6.936, P < 0.0001$	$t_{(33-803)} = -6.873, P < 0.0001$	$t_{(25-590)} = -3.373, P = 0.001$	$t_{(26-928)} = -8.529, P < 0.0001$

All concentrations are calculated as a proportion of the sum of the three components [e.g. %Ca = (Ca/N + P + Ca) × 100]. Statistics are from independent samples *t*-tests comparing the N, P and Ca composition of each food with the composition of the relevant faeces. Positive *t*-values indicate decreased concentration in faeces relative to food, while negative *t*-values indicate increased concentration in faeces relative to food.

whether the food species was *WB* (Fig. 4a) or *AB* (Fig. 4b). This demonstrates that N was extracted from shoots with higher relative efficiency than either P or Ca. Additionally, the Ca : P ratio in faeces was higher than in shoots, indicating that Ca was extracted with lower relative efficiency than P, thus exacerbating the effective deficit of Ca relative to P in shoots. In both species of bamboo, the faeces associated with leaves were relatively enriched in Ca, whereas the proportional concentration of P was statistically unchanged in leaves and faeces (Table 1, Fig. 4a and b).

Discussion

This is the first field study to use nutritional geometry to explore the relationship between the balance of essential nutrients, selection of foraging habitat, and the life cycle of a highly endangered herbivore species. Our data showed pandas experience marked seasonal foraging changes with four primary foraging periods corresponding to the annual phenology of the two bamboo species. The nutritional quality of the diet was heterogeneous through the year, both in terms of the absolute concentrations of N, P and Ca and the proportional balance of these nutrients. Seasonal diet switches corresponded with shifts in quantities of these key nutrients, as did the life cycle, reproduction and pattern of altitudinal migration. As we will discuss, the close correspondence between animal life-history events, shifting range, and forage quality suggest that nutritional balancing is a contributing component that maintains the population of this endangered species, an insight that may be crucial in its conservation as well as that of other endangered species that have narrow dietary ranges.

Our analysis showed that the N–P–Ca composition of diets varied with bamboo species, plant part and the age of the plant part. Age influenced primarily the concentration of nutrients, which declined as both leaves and shoots matured (Fig. 3a and b), most likely due to an increase in plant structural components such as cellulose,

hemicellulose and lignin (Hu *et al.* 1990). In the absence of reliable measures of daily food intake (Rothman *et al.* 2011), which cannot readily be obtained for wild giant pandas in the field, it is difficult to interpret the significance of the concentration of a nutrient in foods in relation to the animal's requirement for that nutrient. Invariably, however, diet switches by pandas corresponded with a change to younger, more nutrient-rich alternatives, whether this involved a different plant part (e.g. arrow 1 in Fig. 3a and b) or species (e.g. arrow 2 in Fig. 3a and b). This preference for younger tissues could relate to their higher nutrient concentrations compared with older tissues in which nutrients are diluted by greater concentrations of structural components. Additionally, the biomechanical properties associated with plant structural components can also reduce the nutritional quality of foods (Clissold *et al.* 2009). Although we did not measure plant-produced allelochemicals, these might play a similar role (Launchbaugh, Provenza & Pfister 2001).

Nutrient concentrations also differed between species of bamboo, but these differences were contingent on the plant part. Specifically, the young shoots of wood bamboo had considerably higher N, P and Ca concentrations than the young arrow bamboo shoots, whereas the species difference was less marked and reversed for leaves (Fig. 3a and b). The greatest difference between plant parts, however, was in the balance of nutrients. Leaves had a higher N : P ratio (Fig. 3a) and a substantially higher Ca : P ratio (Fig. 3b) than did shoots, and this contrast applied for both bamboo species. Such differences in nutrient balance can be a significant parameter of food quality, because nutrient balance determines the ways that nutrients interact in their effects on consumers (Sterner & Elser 2002; Simpson & Raubenheimer 2012).

An important consideration in inferring the functional significance of seasonal diet switches in giant panda is therefore their implications for nutrient balance. Thus, the spring switch by the giant pandas in our study from leaves to shoots of wood bamboo corresponded with increased dietary N content, which could well be an important functional

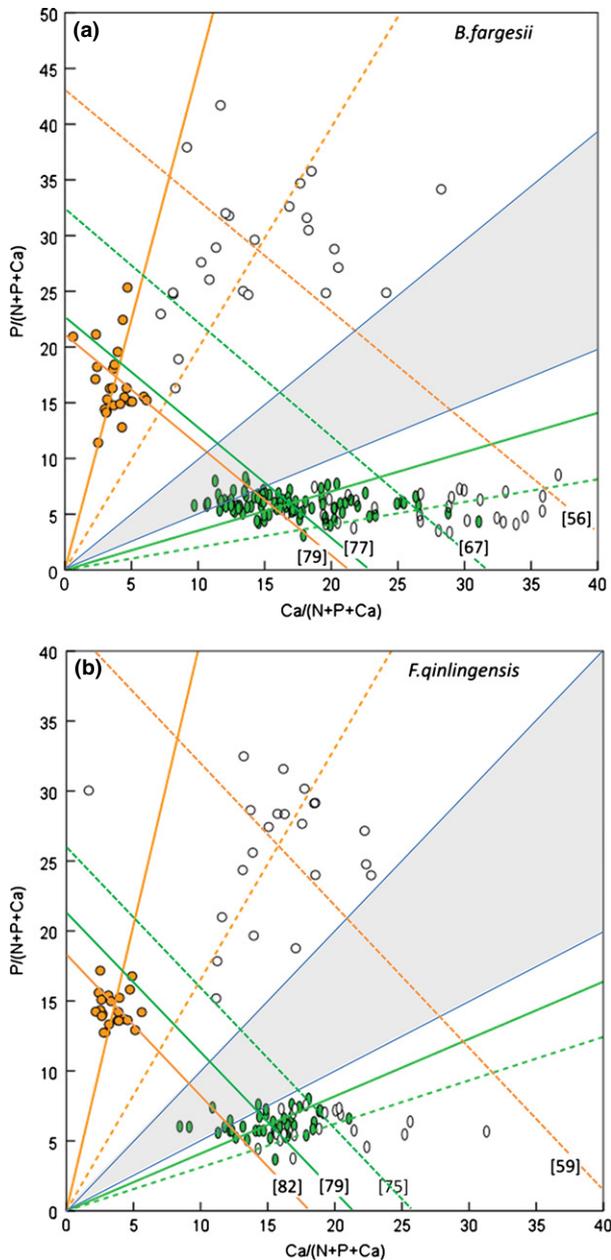


Fig. 4. Comparison of the Ca–N–P composition of foods (shoots and leaves) and the associated faeces in (a) wood bamboo (*WB*) and (b) arrow bamboo (*AB*). Circle-orange = shoots; Circle-empty = shoot-associated faeces; Oval-green = leaves; Oval-empty = leaf-associated faeces. Radials show mean Ca–P ratios, and negatively-sloped diagonals (%N isolines) show the mean percentage of N relative to the sum of N, P and Ca in each sample. Values for the % N isolines are shown in square brackets. Solid lines represent foods (leaves = green, shoots = orange) and dashed lines represent faeces associated with leaves (green) or shoots (orange). The shaded area shows the range of Ca–P ratios recommended in the diets of mammals.

driver of the switch (White 1993). Significantly, dietary P content increased to a proportionally even greater extent than N, and consequently the dietary N : P ratio decreased. Since the N : P ratio of wood bamboo leaves was higher than the optimal range, the decrease corresponding to the

switch to shoots brings the N : P ratio in the diet more in line with estimated requirements (the shaded area in Fig. 3a). The higher P concentrations in the shoots of wood bamboo do, however, have important consequences for giant pandas in relation to the dietary Ca : P ratio.

Dietary Ca : P ratios of 1 : 1–2 : 1 are recommended for mammals (Fig. 3b), with excesses of either nutrient interfering with the absorption and metabolism of the other (Robbins 2001). When the Ca : P ratio drops much below 1, P impedes absorption of the already limiting Ca, resulting in Ca resorption from bones and ultimately osteomalacia (softening of the bones) and associated diseases. In both human and animal studies, Ca : P ratios <0.5 have been associated with reduced bone mass density and compromised bone strength (Calvo & Tucker 2013). These effects can be particularly acute in relation to reproduction because of its increased calcium requirements for lactation and bone growth (Schulkin 2001). It is therefore noteworthy that the high levels of P in young wood bamboo shoots in our study resulted in Ca : P ratios of considerably <1 (0.2). In contrast, Ca–P ratios in leaves were closer to the recommended range for mammals, being marginally above 2 (Fig. 3b). In general, Ca : P ratios higher than 2 (surplus Ca) are tolerated by herbivores to a greater extent than ratios <1 (surplus P) (Robbins 2001).

It is important to note that measures of Ca : P ratios in plant foods might not accurately represent the biologically effective Ca : P ratios, because a proportion of these elements might be bound in molecular complexes that render them unavailable (Suttle 2010). For example, phytic acid is an important storage form of P in many plant tissues (especially seeds) and is poorly digested by non-ruminant herbivores. However, if the relative excess of P in the panda diet was significantly influenced by unavailable P in this way, then, we would expect the faeces would be more highly P-enriched relative to Ca, but this was not the case. Rather, the faeces produced from bamboo shoots were enriched in Ca relative to P (i.e. the Ca : P ratio of faeces was greater than the Ca : P ratio of the shoots), and therefore, the postabsorptive Ca : P ratio associated with shoots was even lower than 0.2 (Fig. 4). The relatively high Ca levels in the faeces are consistent with the interfering effect of surplus P on Ca absorption discussed above. By contrast with shoots, the faeces associated with leaves were enriched in Ca but had statistically similar P contents to the leaves (Fig. 4). This selective egestion of Ca would bring the Ca : P ratio of leaves, which was marginally higher than 2, more closely in line with the recommended range.

Our nutritional analysis therefore implies that, when pandas switch from a diet of old leaves to shoots of wood bamboo at around the time of mating, they shift from a diet that is low in both N and P with a N : P ratio that exceeds the maximum recommended for mammals to a diet that is higher in both nutrients and has an N : P ratio within the recommended range (Fig. 3a). With the subsequent switch to arrow bamboo shoots, the dietary concentrations of

both N and P were reduced but the N : P ratio was very close to the centre of the expected N : P range (c. 6.75, Fig. 3a). These high shoot N and P contents likely help support construction of the placenta and the growing embryo during foetal development. During this period, however, the dietary Ca : P ratio was considerably lower than considered necessary to support reproduction in mammals and was only restored with the subsequent switch to arrow bamboo leaves (Figs 2 and 3b).

These dynamics lead us to suggest that perhaps delayed implantation provides a means for pandas to postpone the Ca investment in lactation and bone growth, synchronizing these more closely with a leaf-based diet that can support them. On the other hand, the relatively low levels of both N and P, and the high N : P ratios in the autumn and winter diet, present additional challenges for panda reproduction. Specifically, both N and P are required for tissue growth, and there would be obvious fitness penalties for pandas that could not acquire these in sufficient quantities for reproduction. An interesting possibility is that this could be related to the evolutionary maintenance and enhancement of the short gestation period of pandas, and the extremely small size of the offspring at birth (Garshelis 2004). Giving birth to altricial young would ease the burden on the mother for acquiring limiting nutrients, by enabling the offspring to start independent feeding (i.e. weaning) earlier. Having both mother and offspring eating to meet their own respective nutrient needs would allow the pair to process bamboo and acquire limiting nutrients at a greater rate than if the burden fell on the mother alone. Barclay (1994) used similar reasoning to argue that the long development time for flight, which delays independent foraging in flying vertebrates (bats and birds), might impose constraints on Ca acquisition for bone growth and explain why these animals generally have small litters. The peak in panda mortality in March and April is also consistent with an interpretation that the extended low quality of the winter diet of leaves is nutritionally stressful, highlighting the need for both mother and offspring to forage for limiting nutrients.

In summary, our analysis has shown that young shoots of wood bamboo were high in P and N but had a Ca : P ratio markedly lower than is considered necessary to support bone growth in mammals. In June, the levels of P, Ca and N dropped in the maturing shoots of wood bamboo, whereupon the pandas migrated to higher elevation. This allowed them to switch to arrow bamboo shoots, which had higher levels of P, N and Ca but again a sub-optimally low Ca : P ratio. By early August, during the late stages of gestation, nutrient levels in arrow bamboo shoots had dropped and the pandas switched to feeding on the leaves of the same species. These had higher nutrient levels, in particular Ca, a more favourable Ca : P ratio, and a better Ca : P absorption profile than bamboo shoots. In August, females returned to the lower elevation feeding sites where birthing coincided with the availability of young wood bamboo leaves, with high nutrient content and a high

Ca : P ratio. However, as wood bamboo leaves aged through the winter, their P content decreased, reaching their lowest level in the period that coincides with the highest historical mortality rate. At that point, the pandas again switched to young wood bamboo shoots once they became available.

Overall, our results also suggest that the two bamboo species are nutritionally interchangeable but that different plant parts (shoots and leaves) are not. Rather, the shoots and leaves are nutritionally complementary resources, with shoots providing primarily N and P, but deficient in Ca, which is provided by the leaves. Both species of bamboo are, nonetheless, critical for the pandas, because their asynchronous phenology, coupled with seasonal altitudinal migration, enables the pandas to complete their life cycle on this low diversity and highly specialized diet. Our insights into the phenological dynamics of panda nutrition have important implications for managing the conservation of this charismatic species in the face of climate change. Tuanmu *et al.* (2012) recently modelled likely future distributions of wood and arrow bamboo in the light of several IPCC climate projections for the Qinling Mountain region (our study area). They noted strong potential for range contraction and elevation shifts in these species, changes that are likely to lead to phenological mismatches between the timing of panda life cycle events and the nutritional suitability of bamboo. Such possibilities highlight the need for a systems-approach to panda conservation, in which pandas, both species of their food plants, and their respective habitats, both current and projected, are all afforded protection. More broadly, this work gives us a new insight into animal nutritional ecology of potential benefit to further research in the field of animal ecology and conservation biology, especially for species that face serious nutritional challenges due to accelerating environmental change.

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References

- Barclay, R.M.R. (1994) Constraints on reproduction by flying vertebrates: energy and calcium. *American Naturalist*, **144**, 1021–1031.
- Bremner, J.M. (1996) Nitrogen: total. *Methods of Soil Analysis. Part 3: Chemical Methods* (eds D.L. Sparks, A.L. Page, P.A. Loeppert, P.N. Soltanpour, M.A. Tabatabai & C.T. Johnston *et al.*), pp. 1085–1123. Soil Science Society of America and American Society of Agronomy, Madison.
- Buchman, A.L. & Moukartzel, A. (2000) Metabolic bone disease associated with total parenteral nutrition. *Clinical Nutrition*, **19**, 217–231.

- Calvo, M.S. & Tucker, K.L. (2013) Is phosphorus intake that exceeds dietary requirements a risk factor in bone health? *Annals of the New York Academy of Sciences*, **1301**, 29–35.
- Clissold, F.J., Sanson, G.D., Read, J. & Simpson, S.J. (2009) Gross vs. Net income: how plant toughness affects performance of an insect herbivore. *Ecology*, **90**, 3393–3405.
- Colles, A., Liow, L.H. & Prinzing, A. (2009) Are specialists at risk under environmental change? Neocological, paleoecological and phylogenetic approaches. *Ecology Letters*, **12**, 849–863.
- Dierenfeld, E., Hintz, H., Robertson, J., Van Soest, P. & Offeda, O. (1982) Utilization of bamboo by the giant panda. *Journal of Nutrition*, **112**, 636–641.
- Elsler, J.J., Dobberfuhl, D.R., MacKay, N.A. & Schampel, J.H. (1996) Organism size, life history, and N: P stoichiometry: toward a unified view of cellular and ecosystem processes. *BioScience*, **46**, 674–684.
- Elsler, J.J., Fagan, W.F., Denno, R.F., Dobberfuhl, D.R., Folarin, A., Huberty, A. *et al.* (2000) Nutritional constraints in terrestrial and freshwater food webs. *Nature*, **408**, 578–580.
- Elsler, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H. *et al.* (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, **10**, 1135–1142.
- Garshelis, D.L. (2004) Variation in ursid life histories: is there an outlier? Comparative ecology of giant pandas in the five mountain ranges of their distribution in China. Chapter 4. *Giant Pandas: Biology and Conservation* (eds D.G. Lindburg, K. Baragona & G.B. Schaller), pp. 53–73. University of California Press, Berkeley, California, USA.
- Goldizen, A.W., Terborgh, J., Cornejo, F., Porras, D.T. & Evans, R. (1988) Seasonal food shortage, weight loss, and the timing of births in saddle-back tamarins (*Saguinus fuscicollis*). *Journal of Animal Ecology*, **57**, 893–901.
- Hu, J., Wei, F., Yuan, C. & Wu, Y. (1990) *Research and Progress in Biology of the Giant Panda*. Sichuan Publishing House of Science and Technology, Chengdu.
- Kuo, S. (1996) Phosphorus. *Methods of Soil Analysis. Part 3: Chemical Methods* (eds D.L. Sparks, A.L. Page, P.A. Loeppert, P.N. Soltanpour, M.A. Tabatabai & C.T. Johnston *et al.*), Soil Science Society of America and American Society of Agronomy, Madison, pp. 869–920.
- Langmyhr, F.J. & Thomassen, Y. (1973) Atomic absorption spectrophotometric analysis by direct atomization from the solid phase. *Zeitschrift für Analytische Chemie*, **264**, 122–127.
- Launchbaugh, K.L., Provenza, F.D. & Pfister, J.A. (2001) Herbivore response to anti-quality factors in forages. *Journal of Range Management*, **54**, 431–440.
- Mayntz, D., Raubenheimer, D., Salomon, M., Toft, S. & Simpson, S.J. (2005) Nutrient-specific foraging in invertebrate predators. *Science*, **307**, 111–114.
- McDowell, L.R. (1996) Feeding minerals to cattle on pasture. *Animal feed science and technology*, **60**, 247–271.
- Milton, K. (1979) Factors influencing leaf choice by howler monkeys: a test of some hypotheses of food selection by generalist herbivores. *American Naturalist*, **114**, 363–378.
- Moen, R.A., Pastor, J. & Cohen, Y. (1999) Antler growth and extinction of Irish Elk. *Evolutionary Ecology Research*, **1**, 235–249.
- Moore, B.D. & Foley, W.J. (2005) Tree use by koalas in a chemically complex landscape. *Nature*, **435**, 488–490.
- Nie, Y., Zhang, Z., Swaisgood, R.R. & Wei, F. (2012a) Effects of season and social interaction on fecal testosterone metabolites in wild male giant pandas: implications for energetics and mating strategies. *European Journal of Wildlife Research*, **58**, 235–241.
- Nie, Y., Swaisgood, R., Zhang, Z., Liu, X. & Wei, F. (2012b) Reproductive competition and fecal testosterone in wild male giant pandas (*Ailuropoda melanoleuca*). *Behavioral Ecology and Sociobiology*, **66**, 721–730.
- Pan, W., Lu, Z., Zhu, X., Wang, D., Wang, H., Fu, D. *et al.* (2001) *A Chance for Lasting Survival*. Peking University Press, Beijing.
- Qiu, Z. & Qi, G. (1989) *Ailuropoda* found from the late Miocene deposits in Lufeng, Yunnan. *Vertebrata Palasiatica*, **27**, 153–169.
- Raubenheimer, D. (2011) Toward a quantitative nutritional ecology: the right-angled mixture triangle. *Ecological Monographs*, **81**, 407–427.
- Raubenheimer, D., Simpson, S.J. & Mayntz, D. (2009) Nutrition, ecology and nutritional ecology: toward an integrated framework. *Functional Ecology*, **23**, 4–16.
- Raubenheimer, D., Simpson, S.J. & Tait, A.H. (2012) Match and mismatch: conservation physiology, nutritional ecology and the timescales of biological adaptation. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **367**, 1628–1646.
- Ritchie, M.E. (2000) Nitrogen limitation and trophic vs. abiotic influences on insect herbivores in a temperate grassland. *Ecology*, **8**, 1601–1612.
- Robbins, C.T. (2001) *Wildlife Feeding and Nutrition, and Edition*. Academic Press, New York.
- Rosenthal, G.A. & Berenbaum, M.R. (1991) Herbivores: their interaction with secondary plant metabolites. *The Chemical Participants*. Vol.1. Academic Press, San Diego, California, USA.
- Rothman, J.M., Raubenheimer, D., Colin, A. & Chapman, C.A. (2011) Nutritional geometry: gorillas prioritize non-protein energy while consuming surplus protein. *Biology Letters*, **7**, 847–849.
- Rubenstein, D.R. & Wikelski, M. (2003) Seasonal change in food quality: a proximate cue for reproductive timing in marine iguanas. *Ecology*, **84**, 3013–3023.
- Sandell, M. (1990) The evolution of delayed implantation. *Quarterly Review of Biology*, **65**, 23–42.
- Schaller, G.B., Hu, J., Pan, W. & Zhu, J. (1985) *The Giant Panda of Wolong*. University of Chicago Press, Chicago, Illinois, USA.
- Schulkin, J. (2001) *Calcium Hunger: Behavioral and Biological Regulation*. Cambridge University Press, Cambridge.
- Simpson, S.J. & Raubenheimer, D. (2012) *The Nature of Nutrition: A Unifying Framework from Animal Adaptation to Human Obesity*. Princeton University Press, Princeton, New Jersey, USA.
- State Forestry Administration, China (2006) *The third national survey report on giant panda in China*. Science, Beijing (in Chinese).
- Sterner, R.W. & Elser, J.J. (2002) *Ecological Stoichiometry: The Biology of Elements From Molecules to the Biosphere*. Princeton University Press, Princeton, New Jersey, USA.
- Suttle, N.F. (2010) *Mineral Nutrition of Livestock*, 4th edn. CAB International, Wallingford, Oxon, UK.
- Thom, M.D., Johnson, D.D.P. & MacDonald, D.W. (2004) The evolution and maintenance of delayed implantation in the mustelidae (Mammalia: Carnivora). *Evolution*, **58**, 175–183.
- Tuanmu, M.N., Vina, A., Winkler, J.A., Li, Y., Xu, W., Ouyang, Z. *et al.* (2012) Climate-change impacts on understory bamboo species and giant pandas in China's Qinling Mountains. *Nature Climate Change*, **3**, 249–253.
- Underwood, E. & Suttie, N. (1999) *The Mineral Nutrition of Livestock*, 3rd edn, pp. 614. CAB International, Wallingford, Oxon, UK.
- Van Soest, P.J. (1994) *The Nutritional Ecology of the Ruminant*, 2nd edn, pp. 476. Cornell University Press, Ithaca, New York, USA.
- Wei, F., Feng, Z., Wang, Z. & Li, M. (1999) Feeding strategy and resource partitioning between giant and red pandas. *Mammalia*, **63**, 417–430.
- Wei, F., Hu, Y., Zhu, L., Bruford, M.W., Zhan, X. & Zhang, L. (2012) Black and white and read all over: the past, present and future of giant panda genetics. *Molecular Ecology*, **21**, 5660–5674.
- White, T.C.R. (1993) *The Inadequate Environment: Nitrogen and the Abundance of Animals*. Springer, New York, USA.
- Zhang, Z.J., Sheppard, J.K., Swaisgood, R.R., Wang, G., Nie, Y.G., Wei, W. *et al.* (2014) Ecological scale and seasonal heterogeneity in the spatial behaviors of giant pandas. *Integrative Zoology*, **9**, 46–60.
- Zhao, S.C., Zheng, P.P., Dong, S.S., Zhan, X.J., Wu, Q. *et al.* (2013) Whole genome sequencing of giant pandas provides insights into demographic history and local adaptation. *Nature Genetics*, **45**, 67–71.
- Zhu, L., Wu, Q., Dai, J., Zhang, S. & Wei, F.W. (2011) Evidence of cellulose metabolism by the giant panda gut microbiome. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 17714–17719.

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