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ORIGINAL PAPER

Ultradian rhythms and the nutritional importance of caecotrophy in captive Brandt's voles (*Lasiopodomys brandtii*)

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Abstract Ingestion of soft faeces derived from caecal contents, caecotrophy, in herbivorous small mammals is considered an adaptation to the metabolic disadvantage of small body size, especially when feeding on diets of low quality. We investigated daily activity patterns in captive Brandt's voles (*Lasiopodomys brandtii*), including feeding, locomotion, caecotrophy, and defaecation, by continuous 24 h visual observation; and estimated the contribution of soft faeces ingestion (caecotrophy) to intake of protein and energy. Brandt's voles ingested 68.8 ± 7.4 fecal pellets per day, averaging 17 ± 2% of total faeces produced. The amount of faeces ingested did not differ between female and male voles or between night and day time. All animals showed average 3 h ultradian cycles in behaviour during the course of the day and night. The contributions of caecotrophy to the dietary intake of crude protein and metabolizable energy were estimated respectively as 9 and 8% on a high-protein, easily digested commercial rabbit pellet diet. However, the importance of caecotrophy to the field voles is likely to be higher on a natural diet of lower nutrient density. The rhythm of caecotrophy in voles depended mainly on the rhythm of the colonic separation mechanism in the proximal colon and passage in the distal colon, and may be regulated by feeding and other activity rhythms. Ultradian rhythms in caecotrophy helped to minimise potential conflicts in utilizing the gut, especially in balancing the caecal fermentation and salvaging nutrients contained in caecal bacteria.

Keywords Brandt's vole · Caecotrophy · Protein intake · Ultradian rhythms

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Introduction

Allometric considerations suggest that small herbivores are inefficient in or incapable of extracting energy from the microbial fermentation of structural carbohydrates (Demment and Van Soest 1985). However, there is much empirical evidence that demonstrates well-developed fibre digestion abilities for a number of small rodent species (e.g. Keys and Van Soest 1970; Hammond and Wunder1991; Pei et al. 2001). A combination of the selective and more rapid passage of fibrous material through the gut, together with changes in gut capacity when energy requirements increase or food quality decreases, may allow small herbivores to escape these allometric constraints (Foley and Cork 1992). The colonic separation mechanism (CSM) found in many small hindgut fermenters allows for the retention of microbes and small, easily digested food particles in the caecum where microbial fermentation and microbial reproduction take place

(Björnhag and Sjöblom 1977; Björnhag 1987, 1994; Björnhag and Snipes 1999; Sperber et al 1983). As a result of the CSM, bacteria and nutrients accumulate in the caecum. However, essential amino acids of microbial origin are not absorbed to any significant extent in the hindgut because of the absence of active transport mechanisms for amino acids (and vitamins) in any region of the hindgut (the caecum, proximal colon and distal colon; Hume et al. 1993).

Almost all small caecal fermenters practise caecotrophy, ingestion of soft faeces derived from caecal contents, thereby utilizing the products of caecal fermentation (Björnhag 1987, 1994; Björnhag and Snipes 1999; Hirakawa 2001). The nutritional significance of caecotrophy was reviewed by Hörnicke and Björnhag (1980) and Stevens and Hume (1995). Only a few researchers have studied the contribution of caecotrophy to protein intake in wild animals (Hörnicke and Björnhag 1980; Chilcott and Hume 1985; Takahashi and Sakaguchi 1998). In smaller rodents (body mass less than 0.1 kg), particularly wild voles, the contribution has not been examined directly, probably because the faeces ingested by lemmings and voles are difficult to distinguish morphologically from discarded faeces. Moreover, although Kenagy and Hoyt (1980) did not find a difference in metabolizable energy content between 'eaten' and 'not eaten' faeces, hindgut fermenters should be able to increase their intake of metabolizable energy by 10–15% by caecotrophy (Alexander 1993).

Similarly, as a result of allometry between body mass and metabolic rate, many microtine rodents exhibit short-term (2–3 h) cycles (ultradian rhythm) of activity and feeding (Lehmann 1976; Daan and Slopsema 1978) to meet their energy needs. Increasing evidence suggests that the ultimate explanation for short-term rhythms in microtines is causally related to specific metabolic and/or digestion needs, probably as a consequence of specialization on bulky and low-energy food resources (Daan and Slopsema 1978; Daan and Aschoff 1981; Zynel and Wunder 2002). In all with caecotrophic microtines so far studied, *Lemmus lemmus* (Björnhag and Sjöblom 1977), *Microtus pennsylvanicus* (Outellettte and Heisinger 1980), *M. californicus* (Kenagy and Hoyt 1980), *M. pinetorum* (Cranford and Johnson 1989), *M. agrestis* and *Clethrionomys glareolus* (Lee and Houston 1993), some faeces are ingested at intervals throughout the day and night, interspersed with periods of eating and drinking. However, the control of the caecotrophy rhythm by exogenous and endogenous factors as well as their ecological significance is not completely understood. Kenagy and Hoyt (1980) suggested that the multiphasic alteration between reingestion and non-reingestion is correlated with adaptation for short-term cycles of activity and feeding in microtines. Cranford and Johnson (1989) suggested that some internal mechanism for digesta separation exists by which specific materials are separated, retained, and periodically released down the colon for reingestion. This mechanism could be the CSM (Björnhag 1987). In addition, the time required for digestion may be the cause for a vole's observed 2–4 h ultradian activity rhythm, given that a vole's stomach capacity limits intake in each feeding bout (Zynel and Wunder 2002). We investigated whether the rhythmicity of caecotrophy is related to ultradian rhythms in feeding and activity.

Brandt's vole (*Lasiopodomys brandtii*) is one of the smallest strictly herbivorous mammals; it feeds on both monocotyledons and dicotyledons, and is distributed primarily in the Inner Mongolian grasslands of China, Mongolia and in the Lake Baikal region of Russia (Zhang and Wang 1998). Brandt's voles do not enter torpor in winter. Instead, they cache substantial amounts of food in autumn and exhibit huddling behavior under cold conditions. Brandt's vole has a large haustrated caecum and complex proximal colon with 2–3 spirals and many oblique folds in the colonic mucosa (personal observations), which is similar to that found in other lemmings and voles (Sperber et al 1983). Pei et al. (2001) found that Brandt's voles have a CSM and confirmed indirectly with a solute digesta marker (Co-EDTA) that Brandt's voles practise faecal ingestion (caecotrophy). The extent and rhythmicity of caecotrophy, and its contributions to protein and energy intake in Brandt's voles are unclear. In this study, we present comprehensive information on caecotrophy in Brandt's voles using an integrative approach, including behavioral strategies and the physiology of the digestive tract in relation to nutritional and ecological factors. We tested our hypothesis that the CSM functions rhythmically and is a cause for the ultradian rhythm of caecotrophy in Brandt's voles.

Material and methods

Animals and diets

All animals in our study came from a breeding colony that started with Brandt's voles that were live-trapped in Inner Mongolian grassland and transported to the Institute of Zoology, Chinese Academy of Sciences in Beijing. They were maintained on commercial rabbit pellets (24.3% crude protein, 25.0% neutral-detergent

fibre (NDF), and 13.6% acid-detergent fibre (ADF), Beijing Ke Ao Feed). Food and water were available ad libitum. All animals used in the experiments were adults housed individually in plastic mouse cages (30 × 15 × 20 cm) under a 12L:12D cycle (lights 08:00 ~ 20:00) and at 22 ± 1°C.

Continuous visual observation of behaviour patterns

The observation apparatus was similar to that used by Kenagy and Hoyt (1980) and Kenagy et al. (1999). We placed animals in a modified plastic mouse box. The bottom of the box was cut out and replaced with a wire screen (5 mm mesh), beneath which was positioned a mirror (80 × 40 cm) at a 45°angle. Eight animals (four females and males, body mass 52.1 ± 1.8 g) were divided into two groups for behavioural observations. Each group consisted of two females and two males. Each group was observed continuously over 27 h. Two of us rotated observational shifts every several hours during the two 27-h studies, including 3 h before the evening light-off to allow animals to become accustomed to the observers. Therefore these 3 h's data were not used for analysis. Seven days before beginning observations, animals were housed singly in a modified box in order to adjust to the box and the dim light (25-W incandescent bulb, filtered through heavy brown paper) at night. We conducted several shorter-period observations at different times on consecutive days to gain first impressions of the existence and nature of caecotrophic behaviour in Brandt's vole. We then made 24-h records of the frequency of caecotrophy (number of soft faeces) and defecation (number of hard faeces), and the time spent in feeding, locomotion, and rest (no spatial moves).

Food intake and defaecation during day and night

We determined day versus night patterns of food intake and faeces deposition for 2 days in metabolic cages by collecting, drying, separating and weighing food residues and faeces just after lights on (0800 h) and just before lights off (2000 h) in another eight Brandt's voles (four females and males, body mass 54.7 ± 5.7 g). All food residues, faeces, and samples of the food were oven-dried to constant mass for 5 days at 60°C. There was no difference in body mass between these and the observed animals ($F = 25.727$, $t = 0.422$, $df = 14$, $P = 0.679$, two-tailed). Collected faeces were counted. Dry mass of a faecal pellet was calculated as the total dry mass of faeces divided by total number of fecal pellets.

Nitrogen concentration of contents of different gut regions

Forty-eight adult Brandt's voles were separated randomly into six groups, and killed at six different times 4 h apart over a 24-h period. The contents of the stomach, caecum, proximal colon, distal colon I (the proximal half) and distal colon II (the distal half) were weighed, oven-dried for 5 days at 60°C to constant mass and assayed for total nitrogen (but content of stomach) by the Kjeldahl method (Foss Kjeltec™ 2100). Crude protein was assumed to be 6.25 times total nitrogen. The gross energy contents of caecal contents were determined using a Parr 1281 oxygen bomb calorimeter (Parr Instrument, USA), with benzoic acid as the standard.

Statistical analysis

Values are presented as means ± 1 SE. Statistical analysis was carried out using the SPSS 13.0 software package for Windows. We used the Wilcoxon signed ranks test to examine differences in measurements between day and night. Mann–Whitney *U* tests were used to determine differences between female and male behavior. Repeated measures analysis of variance (RM-ANOVA) was used to examine changes in nitrogen concentration with time and hindgut region. The Spearman rank-order correlation coefficient was also used to examine relationships between behaviours. A probability value of less than 0.05 was considered significant.

Results

Behavioural patterns and magnitude of caecotrophy

The characteristics of caecotrophy were similar to previous reports on rodent species. Voles bent the head to bring the mouth to the anus, used incisors to pick up faeces from the anus, sometimes with the aid of forelegs, and masticated them well. Jaw movements appeared exaggerated and included a lot of side-to-side motion. The Brandt's voles apparently detected beginning passage of faeces by mainly smelling or tasting. However, most times they ingested faeces or not without any visible detection process during resting, sleeping, and feeding periods. Sometimes, individuals suddenly stopped sleeping (activity or feeding) to ingest two or three pellets in a stance similar to the above, and then continued sleeping (activity or feeding) or did something else. Although several faecal

Table 1 Female versus male faeces deposition and activity timing by Brandt's voles (mean ± SE)

	Female	Male	*P**	Total
Sample size	4	4		8
Body mass (g)	51.7 ± 3.8	52.6 ± 0.6	0.821	52.1 ± 1.8
No. of hard faeces	359.5 ± 27.8	318.8 ± 28.9	0.486	339.1 ± 20.1
No. of soft faeces	71.3 ± 12.9	66.3 ± 9.4	0.686	68.8 ± 7.4
Soft faeces (% of total faeces)	16.6 ± 2.8	17.7 ± 3.6	0.886	17.1 ± 2.1
Feeding (min)	247.3 ± 42.6	212.3 ± 18.4	0.686	229.8 ± 22.5
Locomotion (min)	233.3 ± 60.2	232.8 ± 71.3	1.000	233.0 ± 43.2
Rest (min)	959.5 ± 94.8	995.3 ± 71.7	0.886	977.4 ± 55.4

*Independent-sample *t*-test indicating probability that female and male body mass differ, Mann–Whitney *U* test indicating probability that female and male values differ

Table 2 Daytime versus nighttime faeces deposition and activity timing by Brandt's voles (mean ± SE)

	Night	Day	*P**
Sample size	8	8	
No. of soft faeces	32.9 ± 4.0	35.9 ± 5.5	0.725
Soft faeces (% of total faeces)	18.0 ± 2.21	16.6 ± 2.9	0.674
Feeding (min)	114.9 ± 13.5	114.9 ± 10.9	1.000
Locomotion (min)	145.4 ± 40.0	87.6 ± 29.9	0.183
Rest (min)	459.8 ± 41.5	517.5 ± 32.7	0.161

*Non-parametric test Wilcoxon signed ranks test indicating probability that day and night values differ

pellets were usually passed in succession, a vole took only one of them at a time, waited in an intermediate posture while chewing and swallowing, and then placed the mouth again to the anus to obtain another pellet.

The frequency of caecotrophy per animal per day was not significantly different between males and females (Mann–Whitney *U* test, $P > 0.05$; Table 1), and between day and night (Wilcoxon signed ranks test, $P > 0.05$; Table 2). The voles ingested 17.1 ± 2.1% of total faeces produced, averaging 68.8 ± 7.4 faecal pellets per day.

Rhythms of locomotion, feeding, defaecation and caecotrophy

The Brandt's voles observed continuously over 24 h showed ultradian rhythms similar in period length in activity, feeding (Fig. 1), defaecation and caecotrophy (Fig. 2); there was only weak synchronization among individuals. Times spent feeding, moving (locomotion), and at rest did not differ significantly between males and females (Mann–whitney *U* test, $P > 0.05$; Table 1), or between day and night (Wilcoxon signed ranks test; $P > 0.05$, Table 2).

The frequency of caecotrophy (number of soft faeces ingested per 30 min) and the time spent at rest over 30 min were positively correlated (Spearman correlation coefficient: $r_s = 0.340$, $P < 0.001$, $n = 384$), and were negatively correlated with other behaviours: feeding (time), defecation (number of hard faeces per 30 min) and locomotion (time) ($r_s = -0.231$, -0.405 and -0.265, respectively, $P < 0.001$, $n = 384$). Defaecation correlated positively with feeding and locomotion ($r_s = 0.463$, 0.489, respectively, $P < 0.001$, $n = 384$) and negatively with rest ($r_s = -0.604$, $P < 0.001$, $n = 384$). Moreover, feeding was correlated with locomotion and rest ($r_s = 0.276$ and -0.718, respectively, $P < 0.001$, $n = 384$), and locomotion were negatively correlated with rest ($r_s = -0.798$, $P < 0.001$, $n = 384$). These relationships were consistent with our behavioural observations: Brandt's voles ingested only soft faeces during resting periods but not during feeding and activity periods, while hard faeces emerged mainly during activity and feeding periods.

Food intake and defaecation during day and night

There were no significant differences in food intake and defaecation between day and night in Brandt's voles under the 12L:12D photoperiod (Wilcoxon signed ranks test, $P > 0.05$, Table 3).

Spatio-temporal patterns of nitrogen concentration in hindgut digesta

The concentration of nitrogen in hindgut contents decreased from caecum to distal colon ($F_{3,105} = 117.3$, $P < 0.001$, caecum > proximal colon > distal colon I and II, Fig. 3), suggesting the existence of a colonic separation mechanism (CSM) in Brandt's voles. There was a trend for the concentration of nitrogen to change with time of day ($F_{5,35} = 2.098$, $P = 0.089$, Fig. 3). That may be a result of weak synchrony, because some animals showed the same high nitrogen concentrations of contents in the distal colon as those in caecum, which suggested these faeces were mainly caecotrophes.

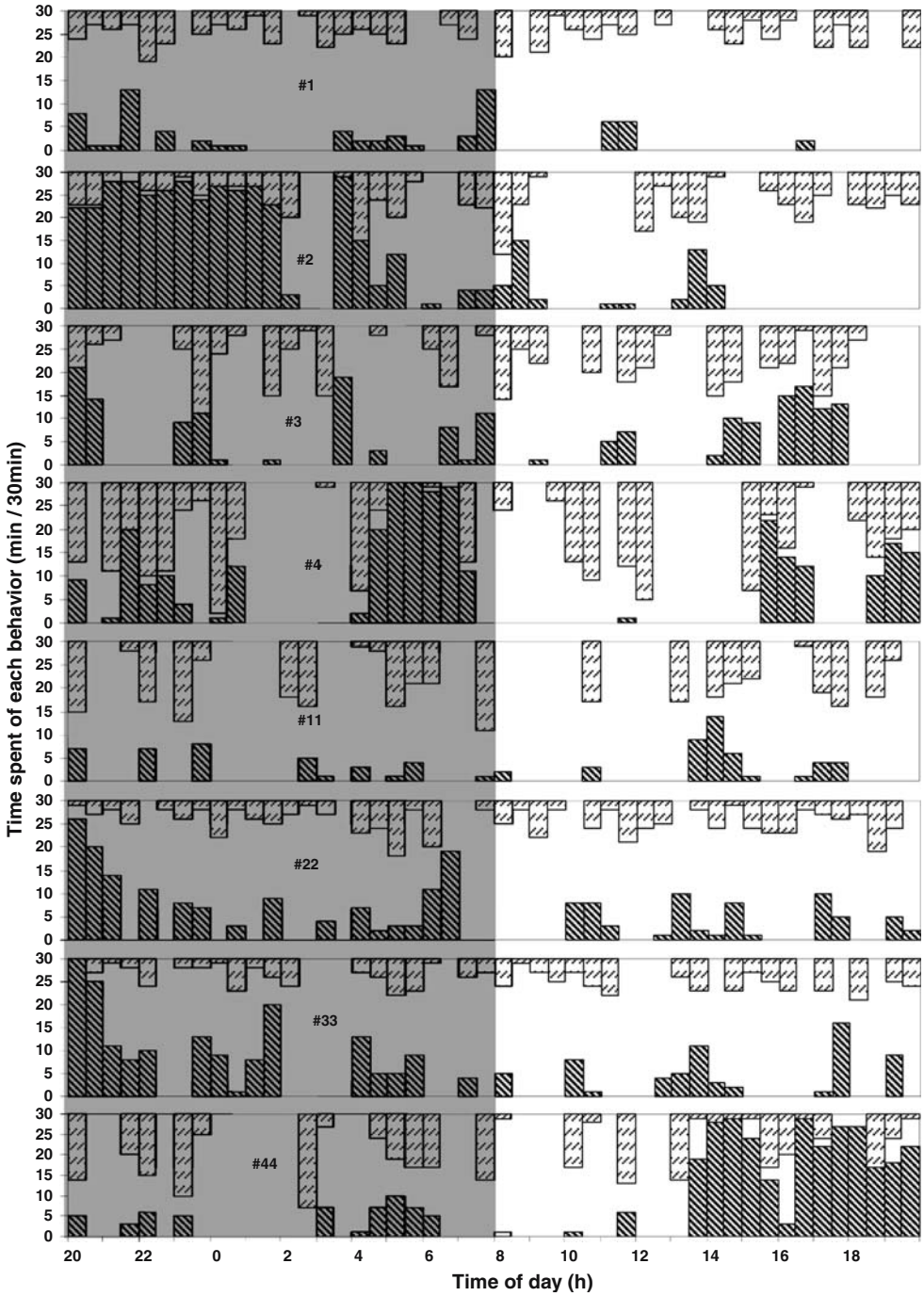

Fig. 1 Daily rhythmic patterns of locomotion (*diagonal bar*, *bottom*), feeding (*dashed bar*, *top*) and rest (*blank*, *mid*) observed continuously for 24 h on a regime of continuous food availability and a photoperiod of 12L:12D, light on 8:00–20:00 hours. Each panel represents a single vole. #1,3,11,33 are male voles, #2,4,22,44 are female voles. Each histogram represents the total behavior of one animal over 30-min periods for a total of 24 h

Estimates of the contribution of caecotrophy to intake

We obtained the total dry mass of soft faeces by multiplying the number of faecal pellets ingested (68.8) for 24 h by the dry mass of a soft faecal pellet, assuming these to be the same mass of a hard fecal pellet (0.0079 g), because the two types of faeces have similar morphology but not nutritional value in microtines (Björnhag, personal communication) and we found no relationship between average dry mass of faecal pellet collected from the distal colon and nitrogen concentration in Brandt's vole ($r_s = -0.038$, $P = 0.732$, $n = 85$). The nitrogen concentra-

Table 3 Daytime versus nighttime food consumption and faeces deposition by Brandt's voles (mean ± SE)

	Night	Day	*P**	Total
Sample size	8	8		8
Food consumption (dry mass, g)	4.38 ± 0.20	4.80 ± 0.28	0.161	9.19 ± 0.38
Faeces deposition[a] (dry mass, g)	1.27 ± 0.13	1.57 ± 0.22	0.069	2.83 ± 0.33
Faeces deposition (n of pellets)	157.8 ± 9.1	192.4 ± 16.4	0.069	350.2 ± 19.7
Dry mass of a pellet (mg)	7.9 ± 0.7	7.9 ± 0.6	0.674	7.9 ± 0.6

*Non-parametric test Wilcoxon signed ranks test indicating probability that day and night values differ

[a] Faeces deposition refers to pellets collected in the metabolic cages, that is, not accounting for faeces that reingested

tion of the faeces ingested was assumed to be same as the average nitrogen concentration (5.23%) of contents in the caecum of all animals killed at different times during the 24 h cycle. In rabbits, rats, water voles, Guinea pigs, chinchilla, nutria and ringtail possum, the nitrogen concentration in soft feces is similar to or greater than that in caecal contents (see Björnhag 1994). Daily dry matter intake was 9.19 g (Table 3), and the food contained 24.3% crude protein on a dry matter basis. Therefore the crude protein intake from food was 2.23 g per day (9.19 × 24.3%). Therefore, contributions of caecotrophy are 5.6% of total dry matter intake and 7.4% of total crude protein intake. However, the protein in soft faeces is easier to digest than that in food (Björnhag 1994). The apparent digestibility of crude protein in this diet by Brandt's voles is 79.5% (Pei et al. 2001). The amount of protein absorbed is therefore 1.78 g per day. Thus, the soft faeces supplied about 9.1% of the total daily intake of crude protein in Brandt's voles.

The mean gross energy of contents in the caecum was 17.68 kJ/g, higher than that in hard faeces and food (16.69 and 17.03 kJ/g, respectively), and was significantly correlated with nitrogen concentration of caecal contents ($r_s = 0.971$, $P < 0.001$, $n = 8$). Using similar calculations, we estimated that soft faeces supplied about 8.2% of the daily metabolizable energy intake.

Discussion

Our observations showed that caecotrophy in Brandt's voles is a normal component of the digestive process, and occurs regularly during resting periods. Caecotrophy contributed about 9.1% of total protein intake and 8.2% of the daily intake of metabolizable energy when voles were fed on commercial rabbit pellets containing 24.3% crude protein.

Behaviour and magnitude of caecotrophy in Brandt's voles

Caecotrophic behaviour in Brandt's voles was similar to that in other rodent species studied, especially other voles (Kenagy and Hoyt 1980; Oullette and Heisinger 1980). Brandt's voles bend the head to bring the mouth to the anus, take soft faeces directly from anus and swallow them after thorough mastication, and caecotrophy occurred regularly during several resting periods in both day and night. This pattern of caecotrophic behaviour may avoid contamination and waste of soft faeces or snatching by other individuals. Moreover, caecotrophy during resting periods can reduce processing conflicts between digestion of soft faeces and food, and increase overall efficiency of gut use (Kenagy et al. 1999). We found that faeces examination seldom occurred in Brandt's voles, which suggested that there must be a sensitive receptor in their rectum that can accurately distinguish soft from hard faeces.

Brandt's voles ate about 17% of total faeces produced, averaging 68.8 ± 7.4 fecal pellets per day. This is similar to findings in captive *Microtus pennsylvanicus* and *M. pinetorum* (Cranford and Johnson 1989). However, the number of faeces eaten by Brandt's voles was more than that in *Dipodomys microps* and *M. californicus* (34 and 28 faecal pellets, Kenagy and Hoyt 1980), although the latter two species ate proportionally more soft faeces (26 and 29%, respectively, Kenagy and Hoyt 1980). The variation in the extent of caecotrophy could reflect the difference in natural or laboratory diet quality (Hörnicke and Björnhag 1980; Kenagy and Hoyt 1980; Cranford and Johnson 1989).

Rhythmicity of caecotrophy

Ultradian caecotrophy rhythms have been reported in *Microtus californicus* (Kenagy and Hoyt 1980), *M. pennsylvanicus* (Ouellette and Heisinger 1980), *M. pinetorum* (Cranford and Johnson 1989), and *Lasiopodomys brandtii* (this study). We estimated that the rhythm duration was 4.03 ± 0.51 h ($n = 3$) for caecotrophy, 2.92 ± 0.29 h ($n = 8$) for feeding, and 3.29 ± 0.48 h ($n = 4$) for locomotion using a chi-square periodogram analysis (Sokolov and Bushell 1978). There were no significant differences among period length of these behaviors, and these periods are similar to the expected value (3.27 h, body mass: 52.1 g) based on

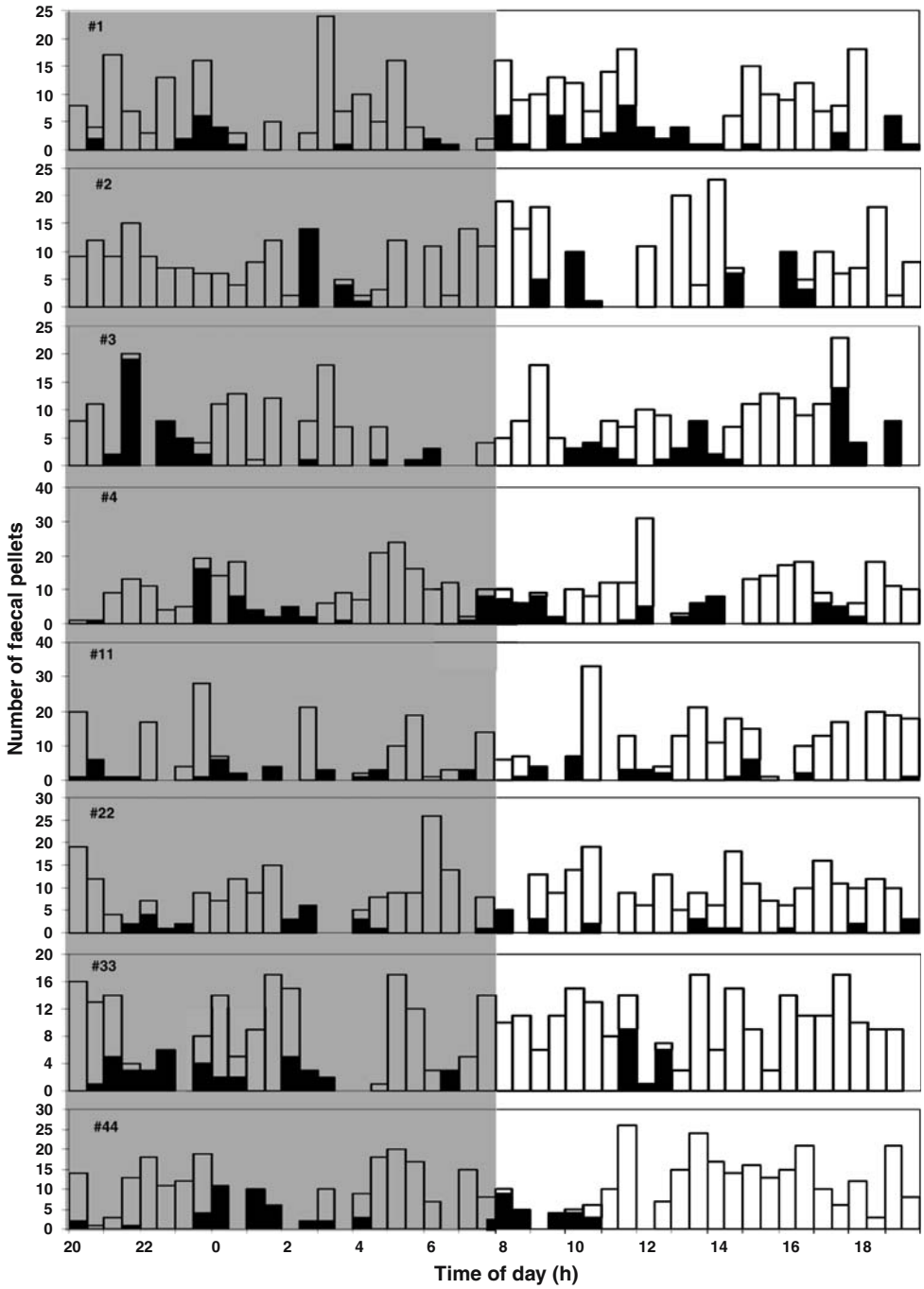

Fig. 2 Daily rhythmic patterns of caecotrophy (*solid bar*) and defecation (*blank bar*) observed continuously for 24 h on a regime of continuous food availability and a photoperiod of 12L:12D, light on 8:00–20:00 hours. Each panel represents a single vole. #1,3,11,33 are male voles, #2,4,22,44 are female voles. Each histogram represents the total behavior of one animal over 30-min periods for a total of 24 h

the formula: $t = 1.00^{*}\ W^{0.30}$ (t in hours, W in gram; Daan and Slopsema 1978). Few data are available on the period of caecotrophy in microtine rodents, so we could not test the relationship between body size and the rhythm period of caecotrophy.

The triggering mechanism of ultradian rhythms must be endogenous, because no external and environmental cues can act as a 'zeitgeber' for the short-term activity pattern (Daan and Aschoff 1981; Halle and Stenseth 1994). Ultradian caecotrophy rhythm is related firstly to forming soft faeces, which occurs at the proximal colon when the CSM ceases for several short periods during both the day and night (Björnhag 1994). However, the behavior of ingesting soft faeces begins only when caecotrophes arrive at the rectum, as observed in all rodents studied (Kenagy and Hoyt 1980; Ouellette and Heisinger 1980; Cranford and Johnson 1989; this study). The CSM may operate again and hard faeces formed while soft faeces pass through the distal colon. Thus, the period of caecotrophy rhythms depends not only on the period of the CSM, but also on the retention time of faecal pellets in the distal colon.

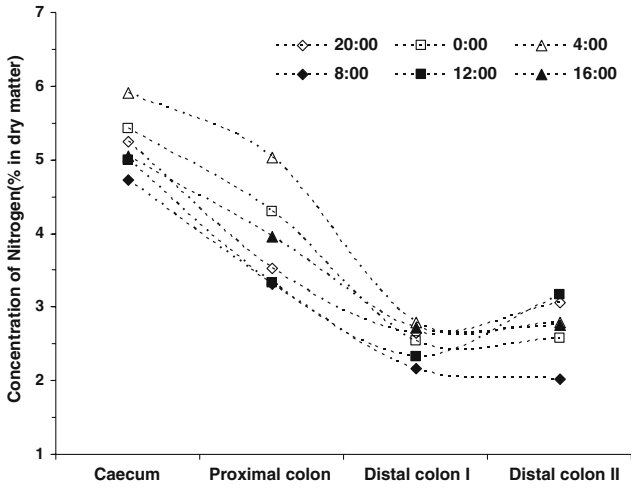

Fig. 3 Concentrations of nitrogen (% of dry matter) in hindgut contents of Brandt's voles

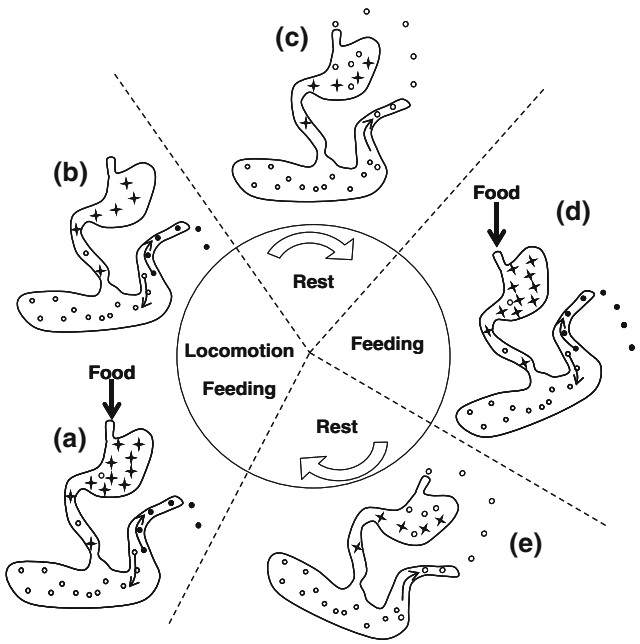

Fig. 4 Short-term cycles of locomotion, rest, feeding, defaecation and caecotrophy in the Brandt's vole. The cycle diagram illustrates the phase transitions between locomotion, resting, feeding/reingesting and defecating, and the excretion of hard and soft faeces in Brandt's voles. The schemata (a–e) around the diagram show the types of digesta, their flow, and the formation and recycling of hard and soft faeces (*cross* digesta, *open circle* soft faeces and caecal material, *solid circle* hard faeces

Figure 4 illustrate the cyclic relationships among feeding, locomotion, rest, defecating, digesta flow and caecotrophy in microtines, based on our observations and the results of other studies. The CSM is activated during feeding and locomotion (Fig. 4a, b, d). Large particles are separated from small particles and solutes in the proximal colon, and pass to the distal colon along the main channel of the proximal colon, and are voided as hard faecal pellets (Björnhag 1994). The solutes and small particles (including bacteria) are retained in the narrow channel of the proximal colon and move back to the caecum where they are mixed with caecal contents. In Brandt's voles, the solute phase marker was retained significantly longer than the large particle phase marker (Pei et al. 2001). Therefore, the operation of the CSM during feeding and activity is of greater benefit when animals eat more food in a meal and concentrate digestive effort in the caecum on the potentially more fermentable fractions of the digesta.

The CSM may cease for several short periods during resting periods (Fig. 4c, e). Caecal contents, including bacteria and nutrients, then pass through the proximal colon and form caecotrophes. When caecotrophes appear at the anus, animals ingest them directly. A pause in the CSM during rest may be advantageous for efficient functioning of the gut. Because the stomach gradually empties after feeding ceases, and nutrients and bacteria are accumulated in the caecum, the pause in the CSM during rest allows these nutrients and bacteria to pass through the proximal colon, to be returned to the stomach via caecotrophy. By this strategy, small herbivorous mammals may avoid temporal and spatial conflicts in functioning of the gut. In this study, we found that the nitrogen concentration of caecal contents was negatively correlated with the dry mass of both gastric contents (Spearman correlation coefficient: $r_s = -0.515$, $P < 0.001$, $n = 47$) and caecal contents ($r_s = -0.634$, $P < 0.001$, $n = 47$), which suggested that the concentration of nutrients and bacteria in caecal contents would increase as digesta left the stomach.

The mechanism of pausing then restarting the CSM is not clear. A gastrocolic or gastrocecal reflex as a result of gastric distension during feeding may stimulate colonic or caecal contractions (see Stevens and Hume 1995), thereby accounting for the relationship between feeding and defecation.

Nutritional and ecological implications of caecotrophy

Although ingested faeces displace food that would normally be taken into the stomach, small hindgut fermenters minimise this conflict by ingesting nutrient-rich soft faeces during resting periods. In Brandt's voles, ingested soft faeces contributed 9.1% of total daily protein intake and 8.2% of total daily metabolizable energy intake, even though the dry mass of soft

faeces eaten was only is 5.6% of total dry matter intake (soft faeces and food). In free-living voles feeding on low-protein dry grass in winter the contribution of ceacotrophy to daily protein intake would be expected to be much higher. For instance, Chilcott and Hume (1985) calculated that in ringtail possums feeding on their natural diet of *Eucalyptus* foliage of 6.9% crude protein content, caecotrophy provided more protein than obtained from the diet. Soft faeces can provide many essential amino acids and vitamins that may be deficient in the diet (Hörnicke and Björnhag 1980; Ebino 1993; Stevens and Hume 1995; Takahashi and Sakaguchi 1998). Caecotrophy in rats plays an important role in the maintenance of intestinal microbial flora (Ebino 1993). Furthermore, it was calculated that preventing caecotrophy would reduce the apparent digestibility of dry matter by 5% in ringtail possums (Chilcott and Hume 1985) and by 5–25% in mountain hares (Pehrson 1983). Thus, caecotrophy provides an avenue to recover nutrients produced by microbial fermentation in the hindgut (Stevens and Hume 1995), and is an important part of the digestion process in small herbivorous mammals (Hirakawa 2001).

Caecotrophy in small herbivorous mammals needs to be in balance with caecal fermentation, since forming soft faeces takes a lot of microorganisms from the caecum. The generation interval of bacteria must on average be 0.69 times their retention time in order to maintain their numbers; the time is about 12.6 h in the bovine rumen microbiota (see Björnhag 1994). In Brandt's voles, the dry contents in the caecum averaged 0.47 ± 0.02 g ($n = 48$), close to the mass of soft faeces ingested daily (0.54 g). Assuming no addition or loss of dry matter during passage through the colon, the mean retention time of dry matter of the soft faeces would thus be about 20.9 h (=0.47 x 24/0.54), which is long enough to maintain the bacterial population. For hard faeces, the retention time was only about 4 h ($=0.47 \times 24/2.8$), which is less than the generation interval of bacteria. Therefore, the extent (by mass) of caecotrophy probably depends on the size of the caecum.

With increasing food intake in response to higher energy demands (cold, growth and lactation) or low-quality diet, most voles enlarge caecal capacity, as seen in *M. ochrogaster* (Gross et al. 1985), *M. oeconomus* (Wang et al. 1995), *M. pinetorum* (Derting and Austin 1998), and *M. brandtii* (Pei et al. 2001; Liu and Wang, unpublished data). However, it is not clear whether voles ingest more faeces and hence more nutrients and energy with increased caecal contents. Flexibility in the extent of caecotrophy in response to variations in energy demand or availability and quality of food is likely to be critical in allowing small herbivores to survive in wild habitats. More intra- and inter-species studies are needed to understand variations in the extent of caecotrophy in response to changes in the nutrient status of the animals.

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