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Nest-building promotes male offspring body weight development and survival in root vole (*Microtus oeconomus*)

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Abstract: Wild root voles (*Microtus oeconomus*) make nests in the field because of the cold winters and the low temperatures in summer nights. When bred in laboratory, most of them continue to make nests. Nevertheless, some root voles do not make nests anymore. To test the hypothesis is that nest-building is an important thermoregulatory behavior to promote the high fitness of the parents even in the normal temperature conditions (at 22±2℃), two experiments were conducted. Part Ⅰ: Thirty mating pairs including fifteen nest-building and fifteen non-nest-building were provided with 5.3 g of cotton to build a nest, for a period of 30 days. The temperatures in nest-building and non-nest-building groups, in and above nest, with animals or without animals, and the changes of temperature during 60 min periods were investigated. Part Ⅱ: Twenty-four broods' were used in this experiment. The body weight on natal day and weaning day, and numbers and age in days of infanticides were recorded. The results showed the average temperature in the nest-building group was significantly higher than the non-nest-building group. On the contrary, average temperature above the nest of nest-building group was lower than for the non-nest-building group. Average body weight on natal day was not different between the nest-building group and the non-nest-building group but average body weight on weaning day was significantly different between the nest-building group and the non-nest-building group. There were significant differences in male offsprings' average body weight between the nest-building group and the non-nest-building group from 2 to 14 days of age. The results indicated that nest-building has significant influence on parents' fitness in root vole bred in laboratory.
Key words: Fitness; Infanticide; Nest-building; Qinghai-Tibeat Plateau; Root vole (*Microtus oeconomus*); Thermoregulation

建巢行为提高雄性根田鼠的个体发育和存活

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摘要：为研究建巢行为对室内繁育根田鼠的可能影响，共设计两个实验：（1）选择 30对配对根田鼠分成两组，即 15对建巢根田鼠（建巢组）和 15对不建巢根田鼠（不建巢组），每对根田鼠提供 5.3 g左右的棉花作巢材，持续 30 d，记录两组有无实验动物时的巢内、巢上温度变化情况。（2）记录 24窝后代的出生时体重、断奶时体重、杀婴的数量和日龄。结果表明，建巢组巢内温度显著高于不建巢组巢内温度，相反的是，建巢组的巢上温度比不建巢组的巢上温度低。两组间后代出生时的平均体重没有显著差异但断奶时的体重存在显著差异。而且，在 2～14日龄间，建巢组和不建巢组雄性后代体重之间存在显著差异。建巢组的杀婴数量极显著低于不建巢组的杀婴数量。因此，建巢行为可以显著提高雄性后代的个体发育和后代的存活。
关键词：根田鼠；建巢行为；适合度；青藏高原；热调节；杀婴行为
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Winter survival of small mammals is facilitated by physiological and behavioral mechanisms that reduce heat loss and increase heat production at low ambient temperatures. Well-built nests provide significant external insulation, offering shelter, and maintaining warm conditions, all of which have significant survival value for rodents exposed to low ambient temperatures. For this reason, a wide variety of rodent species display an increase in nesting activity following cold acclimation (Lynch and Possidente, 1978; Rajendram et al., 1987; Puchalski et al., 1988) that helps them to "anticipate" a decline in temperature during winter.

Nest-building, which is a component of maternal

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behavior is related to the lifetime reproductive success of mice (Bult and Lynch, 1997). A good nest maintains pups at a constant temperature (26℃) before they are able to thermoregulate themselves (Lee, 1973). Indeed, nest-building is affected by a number of factors, such as strain differences (Lee, 1973; Lynch, 1980; Bond et al., 2002), maternal experience (Broida and Svare, 1982), the presence of newborn pups (Gandelman, 1973), hormone levels and genetics (Lisk et al., 1969; Keisala et al., 2007), environmental temperature (Lynch et al., 1976; Lynch and Possidente, 1978) and interactions between these factors all influence nest-building (Broida and Svare, 1982; Carlier et al., 1983; Carlier et al., 1992). In addition to "maternal" nest-building in females, both mouse sexes build nests to be used as a shelter (Moretti et al., 2005) or for thermoregulation (Bult and Lynch, 1997). According to field research, root voles build nests underground to serve as food depositories and to provide warm shelter for themselves and their offspring (Su, 2001). However, whether or not wild voles maintained in the laboratory exhibit nest-building remains unclear. Similarly, we do not know whether nest-building affects the development and survival of offspring in the laboratory.

In our laboratory, we found that some root voles (*Microtus oeconomus*), including both sexes, do not build nests. The probable reason was that the temperature in the animal house (22±2℃) was more comfortable than that in the field (average air temperature: −1.7℃). The present study, therefore, was designed to address the apparent discrepancy in nest-building and non-nest-building groups in root voles living in the laboratory. We hypothesized that nest-building behavior has an important influence on the fitness of root voles even when they live in a constant-temperature environment.

1 Materials and Methods

1.1 Animals, breeding and housing conditions

Wild root voles were captured from a meadow at the Haibei Alpine Meadow Ecosystem Research Station, located on the northeast Qinghai-Tibet Plateau (37°29′—37°45′N, 101°12′—101°23′E). It has a continental monsoon type climate with long, severe winters and short, cool summers. The average air temperature is −1.7℃ with maximum 27.6℃ and minimum −37.1℃. During the winter months, the average air temperature can drop to −15℃ or even −20℃ in the highland areas (Sun et al., 2005).

Subjects were adult root voles that were third generation offspring of field-caught animals, and who had been born and raised under a long day photoperiod (14∶10 h light, dark cycle, lights on at 08∶00 and end at 22∶00 hours Beijing Standard Time). Animals were maintained in clear polycarbonate cages (40 cm×28 cm×15 cm), which contained wood chip bedding and cotton nesting material. Room temperature was 22 ± 2℃ (Zhuo Yi Election Ltd.). Food (BLARC, China) and water were provided ad libitum. Cages were cleaned once a week. All animals were used only once in this experiment.

In order to avoid bacterial infection and other hazards, all cotton was sterilized in 120℃ for 3 h. Sterilized cotton was offered to root voles to build nests in the experiment. All cotton was of comparable weight.

HOBO dataloggers were used to measure the temperatures inside of the nest and outside the nest (non-nest). At the same time, the temperatures 2 cm above the nest and non-nest groups were measured. Separate probes were placed in the permanent shade at 1 cm in the center of the nest without touching the underlay. The temperatures were measured at midday and recorded at 10-second intervals for 10 min.

Animals were weighed using an electronic balance (0.01 g). Litters' average body weight (ABW) on the natal day and weaning day, survival ratio of each brood, number of infanticides and age in days of infanticide were recorded. At the same time, male and female offspring body weights were recorded every 2 days.

1.2 Data analysis

The one-sample K-S test was used to verify the type of distribution of the data. All data on litter development were then analyzed by a paired-sample t-test comparing nest-builders versus non-nest-builders. The temperature differences also were analyzed by paired-sample t-test. Pearson correlation (2-tailed) was used to analyze the relationship within part indices in nest-building and non-nest-building groups, respectively. $P < 0.05$ was significant.

2 Results

2.1 The weight of nest material

Sanitized cotton was chosen as the nest material. Nest materials were weighted for twenty litters in this experiment. The average weight of sanitized cotton in each nest was 5.28 ± 0.16 g.

2.2 Nest-building

In our experiment, not all voles built nests. We determined whether or not nest-building occurred from photos taken on the day after cotton was given. Root voles that did not make nests (non-nest-builders) flattened the cotton in the breeding box by lying on it (Fig. 1A). The offspring then are forced to lie on the cotton wad prior to weaning. However, root voles that nest-build made the cotton into a ball in the corner of the breeding box (Fig. 1B). In this case, root voles lay under the cotton blanket most of time, especially when asleep. As a result, the offspring are maintained in good heat-preservation conditions.

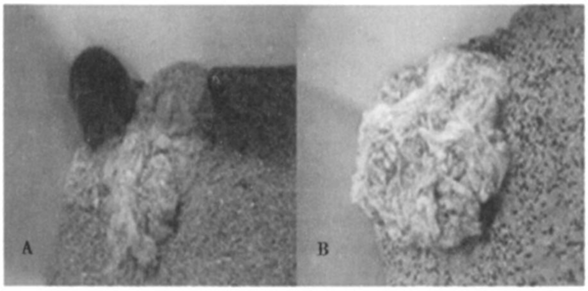

Fig 1 A: Photo of root vole that did not make a nest (non-nest-building). B: Photo of root vole nest (nest-building).

2.3 The temperature inside of the nest

Average temperature for inside nests (ATIN) was significantly higher than average temperature for at the surface for non-nest-builders (ATINN, Table 1). In contrast, average temperature above the nest in nest-building (ATAN) was lower than average temperature above the nest in non-nest-building (ATANN, Table 1).

Table 1 Comparison of average temperature above and within or at the surface for nest-building and non-nest-building mice respectively (Mean±SE)

Group	Mean±SE (℃)	df	T-value	Sig
ATIN	26.07±0.65	11	14.705	P<0.01
ATINN	23.81±0.90	11		
ATAN	22.83±0.13	11	−18.027	P<0.01
ATANN	24.29±0.69	11		

ATIN: average temperature in nest-building; ATINN: average temperature in non-nest-building; ATAN: average temperature above nest in nest-building; ATANN: average temperature above nest in non-nest-building

In order to test the heat preservation function of the nest, the temperature in the nest and above the nest were recorded. This difference remained consistent across time, with ATIN temperatures being significantly higher than ATAN ones from the beginning to the end of the 600 s sample period (Fig 2). For both ATIN and ATAN, temperature declined after the animals had been removed (at the beginning), but temperatures declined significantl faster in the ATAN condition than in the ATIN condition (Fig 2).

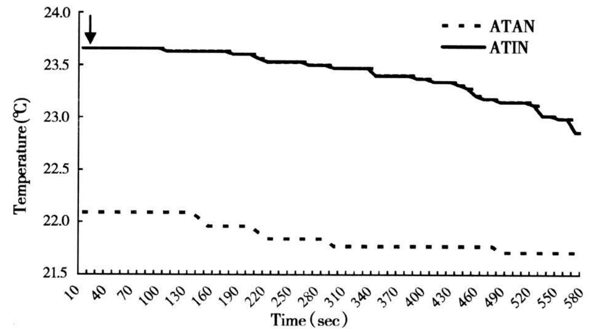

Fig 2 Average temperature decline curves in and above the nest for the nest-building group ATIN: average temperature in nest; ATAN: average temperature above nest. The arrow indicates that the root voles were removed at this point

2.4 Reproductive indices

Key reproductive indices for the two groups are summarized in Table 2. There was no difference in litter size between nest-building and non-nest-building mice (P>0.05). Moreover, the difference in average body weight (ABW) between the nest-building group and the non-nest-building group was not significant (P>0.05). However, ABW on weaning day was significantly higher in the nest-building group than in the non-nest-building group (P<0.05). The survival ratio in the nest-building group was significantly higher than that in the non-nest-building group (P<0.01), while the number of infanticides was lower in the nest-building group than in the non-nest-building group (P<0.01). However, there was no difference between the groups in the mean age of infanticide victims (P>0.05).

Table 2 Reproductive indices for offspring in nest-building and non-nest-building groups (Mean±SE)

Index	Nest-building	Non-nest-building	T-value	Sig
Litter size (No.)	5.25±0.31	4.78±0.23	1.359	P>0.05
Natal ABW (g)	2.24±0.04	2.22±0.03	0.314	P>0.05
Weaning ABW (g)	11.04±0.46	7.99±1.39	2.160	P<0.05
Survival ratio	0.67±0.05	0.34±0.07	3.984	P<0.01
Number of infanticides	1.72±0.27	3.41±0.38	−3.996	P<0.01
Age of infanticide victims (days)	7.65±0.93	7.66±1.08	−0.011	P>0.05

We recorded the body weight of male and female voles in the two groups every other day and compared them using a paired-sample t-test. The difference was significant for male offspring between 2 to 14 days of age (df=22, Fig 3a), but not for female offspring (df=22, Fig 3b).

Tables 3 and 4 give pairwise correlation coefficients between the various reproductive indices for the two nest-building groups. In the nest-building group, there was a significant negative correlation between litter size and natal ABW, while litter size and number of infanticides, and litter size and litter order were significantly positively correlated (Table 3). Survival ratio was significantly negatively correlated with number of infanticides

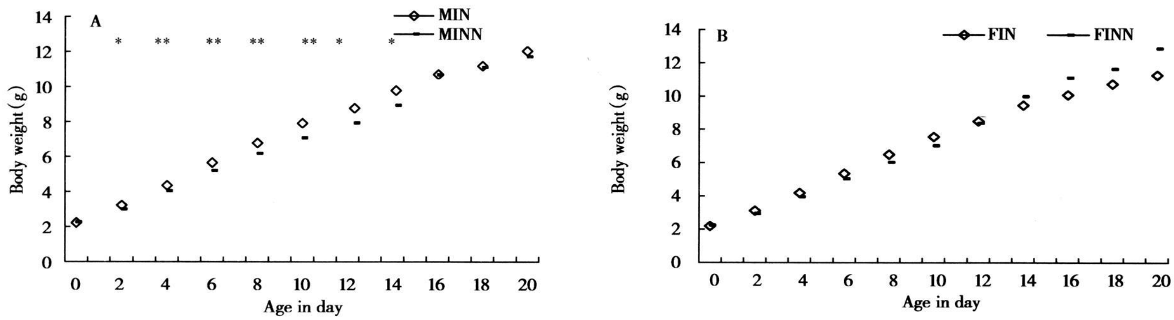

Fig. 3 Comparison of body weight across the first 20 days of life for (a) male root voles and (b) female root voles for nest-building group and non-nest-building group. MIN: males in nest-building; MINN: males in non-nest-building; FIN: females in nest-building; FINN: females in non-nest-building. * indicates there was significant difference between two groups for a given day (P < 0.05). ** indicates there was very significant difference between two groups (P < 0.01).

Table 3 The correlation of some indices in the nest-building group

		Natal ABW	Weaning ABW	Survival ratio	Number of infanticides	Age at infanticide	Litter order
Litter size	PC	−0.553**	−0.215	0.060	0.372*	0.078	0.405*
	Sig	0.001	0.264	0.743	0.036	0.707	0.022
Natal ABW	PC		0.292	−0.331	0.024	−0.108	−0.228
	Sig		0.125	0.064	0.895	0.600	0.209
Weaning ABW	PC			−0.087	0.056	−0.088	0.156
	Sig			0.654	0.772	0.688	0.419
Survival ratio	PC				−0.851**	0.064	−0.041
	Sig				0.000	0.758	0.822
Number of infanticides	PC					−0.033	0.188
	Sig					0.873	0.304
Age at infanticide (days)	PC						0.058
	Sig						0.777

ABW: Average body weight; PC: Pearson Correlation. ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

Table 4 The correlation of some indices in the non-nest-building group

		Natal ABW	Weaning ABW	Survival ratio	Number of infanticides	Age at infanticide	Litter order
Litter size	PC	−0.752**	−0.227	−0.359*	0.600**	−0.258	0.159
	Sig	0.000	0.246	0.044	0.000	0.184	0.384
Natal ABW	PC		0.189	−0.029	−0.204	0.130	−0.224
	Sig		0.335	0.874	0.262	0.510	0.218
Weaning ABW	PC			0.733**	−0.603**	0.056	0.109
	Sig			0.000	0.001	0.794	0.580
Survival ratio	PC				−0.928**	0.273	0.090
	Sig				0.000	0.159	0.624
Number of infanticides	PC					−0.348	−0.044
	Sig					0.070	0.811
Age at infanticide (days)	PC						−0.569**
	Sig						0.002

ABW: Average body weight; PC: Pearson Correlation. ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

In the non-nest-building group, there were significant negative correlations between litter size and natal ABW, and between litter size and survival ratio (Table 4) and there was a significantly positive correlation between litter size and number of infanticide (Table 4). ABW in weaning day has positively correlated with survival ratio and negatively correlated with number of infanticides (Table 4). Survival ratio was significantly negatively correlated with litter size and number of infanticides, but significantly positively correlated with weaning ABW. There was a positive correlation between number of infanticides and litter size, whereas there was a negative correlation between number of infanticides and weaning ABW, and between number of infanticides and survival ratio. There was also a significant negative correlation between age at infanticide and litter order.

3 Discussion

Behavioral adaptations are generally considered to be the principal mechanisms that enable homeotherms to cope with various stresses in their environment. An obvious example is nest-building behavior (Wunder, 1984), which helps to reduce the rate of heat loss from an animal inside its nest and minimize the amount of energy required to maintain homeothermy (Vogt and Lynch, 1982). It is essential that the females prepare high quality nests at the appropriate time to ensure the well-being and survival of the hairless pups. Root voles are hairless when born. So, whether parents build nests or not will have a very important effect on baby root vole survival and development. In our experiment, nest-building increased ATN (26.07 ± 0.65℃) and decreased ATAN (22.83 ± 0.13℃) compared with non-nest-building. Also, the rate of temperature drop was significantly slower in the nest-building group than in the non-nest-building group when the root voles were removed from the nest. So, our research indicated that nest-building plays an important role in preventing heat loss (Table 1 and Fig. 2).

Temperature is an important environmental cue for thermogenesis in root voles. It has been reported that cold exposure induced increases in thermogenesis with decreases in body mass (Wang et al., 1996, 1999). Bult and Lynch (1997) found that nest-building was positively correlated with offspring survival and quality at 4 and 22℃ in mice that were bidirectionally selected for thermoregulatory nest-building behavior. Similarly, rabbits that fail to prepare a maternal nest before parturition usually give birth outside the box provided for nesting, and generally are not successful in raising their offspring (Negatu and McNitt, 2002). In this experiment, there were no differences in litter size, natal ABW and age at infanticide between the nest-building and non-nest-building groups. Weaning ABW and survival ratio were significantly higher in the nest-building group than in the non-nest-building group, while the number of infanticides was lower in nest-building groups than in non-nest-building groups. These results suggest that nest-building promotes the development and survival of offspring, but had no influence on litter size, natal ABW or age at infanticide (Table 2 and Fig. 3). In our experiment, parents in the non-nest-building condition with at low temperatures faced considerable difficulty in rearing pups even though food was more than sufficient and temperatures were stable (22.2℃). However, temperatures were much lower in this condition than was desirable and was the main reason for the high mortality of their offspring. Thus these findings suggest that there is a significant fitness advantage for nest-building phenotypes.

Finally, the correlation analyses suggested that there were some distinct differences between the nest-building and non-nest-building groups. Survival ratio was affected by litter size and weaning ABW, but not the number of infanticides in the non-nest-building groups. However the number of infanticides was negatively correlated with weaning ABW, while age at infanticide was negatively correlated with litter order in the non-nest-building group. All of these results suggest that better nests enhanced the fitness of parents. In *Mus domesticus*, Bult and Lynch (1997) found that in all lines the production and survival of offspring was substantially reduced at 4℃ compared to 22℃, but the high-selected lines produced more and better-quality offspring, surviving up to 40 days of age at both temperatures compared to the control and low-selected lines. The result suggested that thermoregulatory nest-building behavior and evolutionary fitness are closely associated.

In summary, nest-building does promote the development of offspring. And it has very important effects on the fitness of parents. Our research supports the hypothesis that nest-building is very necessary for root voles, and they display this necessary behavior even when bred in laboratory.

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References:

Bolazzi M, Roces F. 2007. To build or not to build: circulating dry air organizes collective building for climate control in the leaf-cutting ant *Acromyrmex ambiguous*. Animal Behavior, 74: 1349－1355.

Bond T L Y, Neumann P E, Bruce Mathieson W, Brown R E. 2002. Nest building in nulligravid, primigravid and primiparous C57BL/6J and DBA/2J mice (*Mus musculus*). Physiol Behav, 75: 551－555.

Broida J, Svare B. 1982. Strain-typical patterns of nest building in mice: maternal and experiential influences. Physiol Behav, 29: 153－157.

Bult A, Lynch C B. 1997. Nesting and fitness: lifetime reproductive success in house mice bidirectionally selected for thermoregulatory nest-building behavior. Behav Genet, 27: 231－240.

Carlier M, Roubertoux P, Cohen-Salmon C. 1983. Early development in mice: I. Genotype and post-natal maternal effects. Physiol Behav, 30: 837－844.

Carlier M, Nosten-Bertand M, Michard-Vanhee C. 1992. Separating genetic effects from maternal environmental effects. In: Goldowitz D, Wahlsten D, Wimer R E. eds. Techniques for the genetic analysis of brain and behavior. New York: Elsevier, USA, 111－120.

Ellison G T H. 1995. Is nest building an important component of thermoregulatory behaviour in the pouched mouse (*Saccostomus campestris*)? Physiol Behav, 57: 693－697.

Fahri L E. 1987. Exposure to stressful environments. In: Dejours P ed. Comparative Physiology of Environmental Adaptations, 2 vol., 8th

ESCP Conference. Strasbourg. Basel. Karger
Gandelman R. 1973. Induction of maternal nest building in virgin female mice by the presentation of young. Horm Behav 4: 191－197.
Gordon C J, Fehler K S, Long M D. 1986. Relationship between autonomic and behavioural thermoregulation in the golden hamster. Am J Physiol 251: R320－R324
Keisala T, Minasyan A, Jarvelin U, Wang J, Hamalainen T, Kalueff A V, Tuohimaa P. 2007. Aberrant nest building and prolactin secretion in vitamin D receptor mutant mice. J Steroid Biochem and Molecul Biol 104: 269－273
Lee C T. 1973. Genetic analyses of nest-building behavior in laboratory mice (Mus musculus). Behav Genet 3: 247－256
Lisk R D, Pretlow R A, Friedman S M. 1969. Hormonal stimulation necessary for elicitation of maternal nest-building in the mouse (Mus musculus). Anim Behav 17: 730－737.
Lynch C B. 1973. Environmental modification of nest-building in the white-footed mouse. Peromyscus leucopus. Anim Behav 22: 405－409.
Lynch C B. 1980. Response to divergent selection for nesting behavior in Mus musculus. Genetics 96: 757－765.
Lynch C B, Possidente B P. 1978. Relationships of maternal nesting to thermo-regulatory nesting in house mice (Mus musculus) at warm and cold temperatures. Anim Behav 26: 1136－1143.
Lynch G R, Lynch C B, Dube M, Allen C. 1976. Early cold exposure: effects on behavioral and physiological thermoregulation in the house mouse. Mus musculus. Physiol Zool 49: 191－199.
Moretti P, Bouwknecht J A, Teague R, Paylor R, Zoghbi H Y. 2005. Abnormalities of social interactions and home-cage behavior in a mouse model of Rett syndrome. Hum Mol Genet 14: 205－220.
Negatu Z, McNitt J I. 2002. Hormone profiles and nest-building behavior during the periparturient period in rabbit does. Anim Reprod Sci 72: 125－135.
Puchalski W, Bulova S J, Lynch C B, Lynch G R. 1988. Photoperiod, temperature and melatonin effects on thermoregulatory behaviour in Djungarian hamsters. Physiol Behav 42: 173－177.
Rajendram E A, Brain P F, Parmigiani S, Mainardi M. 1987. Effects of ambient temperature on nest construction in four species of laboratory rodents. Boll Zool 54: 75－81.
Su J P. 2001. Comparison of the habitant choice between plateau pikas (Ochotona curzoniae) and Gansu pikas (O. cansus). Ph D. thesis. Northwest Plateau Institute of Biology, Chinese Academy of Science.
Sun P, Zhao X Q, Klein J A, Wei W H. 2005. Local warming about 1.3℃ in alpine meadow has no effect on root vole (Microtus oeconomus) population during winter. Pol J Ecol 53: 123－127.
Vogt F D, Lynch G R. 1982. Influence of ambient temperature, nest availability, huddling and daily torpor on energy expenditure in the white-footed mouse Peromyscus leucopus. Physiol Zool 55: 56－63.
Vishalakshi C, Singh B N. 2008. Effect of developmental temperature stress on fluctuating asymmetry in certain morphological traits in Drosophila ananassae. J Therm Biol 33: 201－208.
Wang D H, Sun R Y, Wang Z W, Liu J S, Chen Z. 1996. Adaptive thermogenic properties during cold exposure in root voles (Microtus oeconomus). Acta Zool Sin 42: 369－376.
Wang D H, Sun R Y, Wang Z W, Liu J S. 1999. Effects of temperature and photo-period on thermogenesis in plateau pikas (Ochotona curzoniae) and root voles (Microtus oeconomus). J Comp Physiol B 169: 77－83.
Wunder B A. 1984. Strategies for and environmental cueing mechanisms of seasonal changes in thermoregulatory parameters of small mammals. In: Merritt J F ed. Winter ecology of small mammals. Speccial Publication of Carnegie Museum of Natural History, 165－172