

Overwintering in nests on the ground in the harvest mouse

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Abstracts The harvest mouse *Micromys minutus* has adapted to inhabit the stalk zone of grassland vegetation and is identified by the characteristic nests this species builds on grasses above the ground. Such aerial nests have been considered as an almost exclusive sign of harvest mouse populations and have therefore also been used to determine habitat choice and population density of the harvest mouse. However, we found that nests built on the ground occur after farmers burn away grasslands dominated by native grass *Miscanthus sinensis* and *Pleioblastus chino*. The aim of this study was to determine whether the harvest mouse habitually builds this type of nest and, if so, when it occurs. Because it was difficult to locate nests on the ground when the soil was covered with grasses, we located all the nests in the native grasslands after burning in the spring in 2 consecutive years. We then attempted to estimate nesting time by comparing the mineral content of nest materials to mineral dynamics in *M. sinensis* leaves. Nests were distributed over all the grasslands investigated, and nesting times were calculated to occur between early September and middle March. Our findings demonstrate the harvest mouse repeatedly constructs this type of nest for overwintering. Considerable nondetection errors would have occurred with regard to this species by exclusively interpreting data on the presence or absence of aerial nests.

Keywords *Micromys minutus* · Nest · *Miscanthus sinensis* · Controlled burning · Grassland management · *Pleioblastus chino*

Introduction

The harvest mouse *Micromys minutus* Pallas is highly adept at climbing grass leaves; it is remarkably small (adults weigh 7–9 g) and grasps the grass leaves and stems with its hands and feet and a prehensile tail and shows that *M. minutus* has effectively adapted to and inhabited the stalk zone of gramineous vegetation (Ishiwaka and Mori 1999). This species is widely distributed in temperate and humid climate zones located between east Asia and west Europe (Harris 1991), where most herbaceous vegetation shifts to climax forest, except in areas where disturbances such as fires, floods, grazing, and harvesting occur. Despite such a wide distribution of the species, the International Union for Conservation of Nature (IUCN) has listed it in the near-threatened category since 1996 (IUCN 2006).

In England, Gramineae was used for nest building in more than 90% of cases in which the grass species used had been identified (Harris 1979). *M. minutus* commonly builds characteristic globular nests measuring 5–9 cm in diameter in the stalk zones of gramineous vegetation. Such aerial nests may be suspended between several vertical grass stems or supported between stems that have been laid flat by outside agents (Trout 1978b). The framework of a nest is composed of 20–30 live grass leaves or leaf sheaths, with which the nests are suspended 10–200 cm above the ground (R. Ishiwaka, personal observation). The nests of harvest mice are composed of 3 or 4 layers, each handled in a different manner (Shiraishi 1969; Trout 1978b). The nest materials used for nest building are torn along the leaf

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veins and neatly woven. Toward the interior of the nest, the width and length of the materials progressively decreases (Shiraishi 1969). The nests are very specific, and nest density has often been used as an index of the number of harvest mice in a particular area (Trout 1978b; Bence et al. 2003; Surmacki et al. 2005).

We retrieved nests from the bottom of the vegetation after harvesting or controlled burning in grasslands. Certain features of these nests revealed that they were built by *M. minutus*. We determined they had been built on the ground and had not fallen from the stalk zone for 2 reasons: they retained their globular shape, and most were found in the centers of grass clumps where aerial nests rarely fall. It has been reported that the harvest mouse builds nests on the ground or under surface litter layer (Shiraishi 1969; Banks 1973; Trout 1978b). In this paper, we refer to nests on the ground as “bottom nests” and to those in the upper layer of vegetation as “aerial nests” or “suspended nests” (Warner and Batt 1976; Trout 1978a, b). Systematic investigation of bottom nests have not been reported, probably owing to the difficulty in finding such nests under the vegetation cover, but analyses of these nests will lead to a deeper understanding of the nesting ecology of the harvest mouse. The aims of this study were to demonstrate that the harvest mouse habitually builds some nests on the ground and to clarify function of the nests by estimating when they were built. We discussed factors that would have effects on the density of bottom nests after burning as well as a role of controlled burning as habitat management.

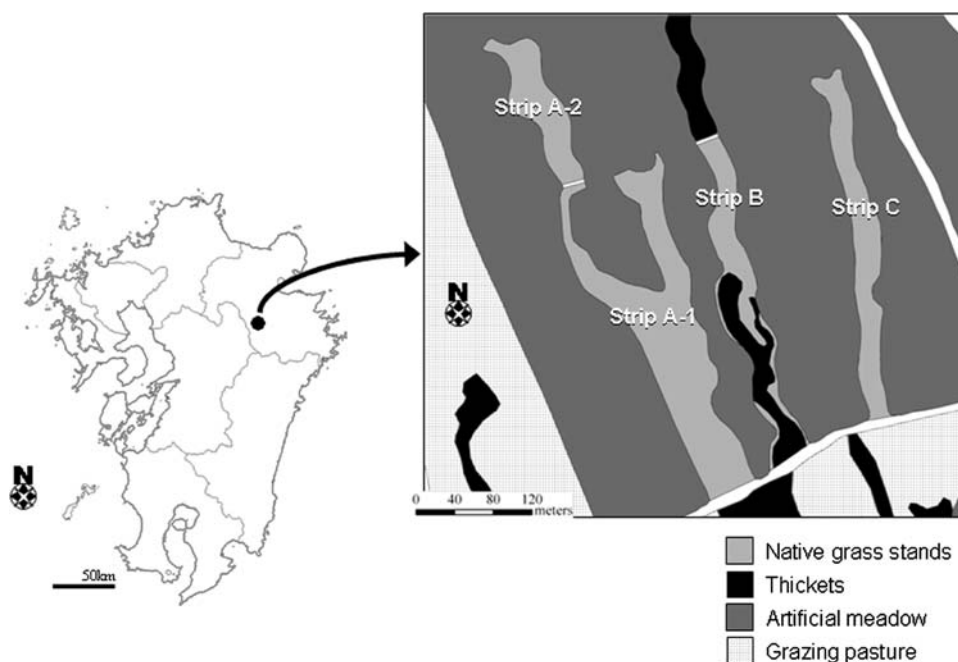
Retrieval of the bottom nests while the ground is still covered with vegetation is a laborious task. Therefore, we located and sampled bottom nests left unburned or

carbonized in grasslands after controlled burning in spring and attempted to determine when the nests were built by studying their mineral content in relation to the mineral dynamics in the leaves. In general, mineral content in plants changes seasonally. We analyzed the leaves of *M. sinensis* Andersson var. *sinensis*, a dominant plant species frequently used for nest building by the harvest mouse in our study area. *M. sinensis* is a native gramineous and perennial species found in Japan, which flowers in autumn, and parts of the plant body located above the ground wither away in winter.

Methods

We conducted our study in the agricultural grasslands at Kuju, Kyushu, Japan (lat 131°23'E, long 33°04'N, 830 m above sea level), which include artificial meadows, grazing pastures, linear native grass stands dominated by *M. sinensis* and *Pleioblastus chino* (Franch. et Savat.) Makino var. *vaginatus* (Makino) S. Suzuki, and linear thickets; hereafter both linearly shaped areas are referred to as “strips” (Fig. 1). *M. sinensis* and *P. chino* were dominant in strip A-1 (1.96 ha), and only *M. sinensis* was dominant in A-2 (0.57 ha) and C (0.86 ha). The central area of strip B (1.03 ha) was dominated by both *M. sinensis* and *P. chino*, and the northern and southern parts were covered with thickets, including approximately 30 plant species. Each strip traversed an artificial meadow dominated by *Lolium multiflorum* Lam. from November to July and *Digitaria adscendens* (H.B.K.) Henrard and *Echinochloa crus-galli* (L.) Beauv. from August to October. The

Fig. 1 The grassland landscape in which the investigation on bottom nests of the harvest mouse was carried out. This agricultural landscape comprises artificial meadows, grazing pastures, linear native grasslands and thickets (strips), and roads



meadows are harvested in May, July, and October every year. The farmers burn strips A-1 and C in spring; in April 2005, strip A-2 and the central and southern areas of strip B were also burned. Farmers said that strips A-2 and B were burned for the first time in several years; strip B had not been burned for more than 10 years.

Immediately after controlled burning was carried out, we conducted a survey on strips A-1 and C in April 2004 and strips A-1, A-2, B, and C in April 2005, respectively. We recorded the location of all bottom nests using a GPS device (Pathfinder Pro XR, Trimble). In both years, the relationship of bottom nest distribution with height, inclination, and direction of the slope was assessed by multiple regression analysis. Topographical measurements were made using a digital surface model (DSM) based on images obtained from the Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) sensor (geometric resolution 15 m) on the Terra satellite. Vegetation in the strips, except for thickets in strip B, were classified according to the 2 dominant plant species *M. sinensis* or *P. chino* based on stepwise discriminant analysis of ASTER images acquired from September 2001 to January 2004 (accuracy of discrimination 70.2%). We used chi-square analysis to compare nest distribution with the abundance of *M. sinensis* or *P. chino* in each year. The number of nests in the pixels in which *M. sinensis* was dominant was compared with the number of pixels in which *M. sinensis* was dominant as the expected values, and the number of nests in the pixels in which *P. chino* was dominant was compared to the number of pixels in which *P. chino* was dominant as the expected values. With regard to strip B, a section of the 1:20000-scale aerial photograph taken above this strip in 2005 was visually analyzed to distinguish pixels dominated by trees. The bias of nest distribution toward plant types (trees or grasses) in the grid was determined with chi-square analysis using the numbers of pixels in which each plant type was dominant as the expected value. During the search of the nests, carcasses that could be identified with the naked eye were found and recorded.

Meteorological data at Taketa, Oita AMeDAS Station (lat 131°22.8'E, long 32°58.4'N, 290 m above sea level, and 9.5 km away from our study area), which is the closest station to our study site, were analyzed using Climate Statistics (Japan Meteorological Agency 2009). The number of snowy days and maximum snow depth in each month at Asosan Meteorological Station (lat 131°04.4'E, long 33°52.8'N, 1142.3 m above sea level, and 23.0 km away from our study area) were obtained. This station was the closest one where snowfall is recorded (Japan Meteorological Agency 2009).

We sampled bottom nests at random in both years. In 2004, 6 nests were obtained from strip A-1 and 5 from strip

C; whereas in 2005, 5 and 4 nests were obtained from strips A-1 and C, respectively. The samples were maintained at approximately 4°C. Materials from the upper surface of the nest interior were analyzed, as this particular area would not have been exposed to rain or contaminated by urine and feces of the former inhabitants. Objects other than leaves that had been used as nest materials, such as florets or insect bodies, were removed to prevent contamination. Sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), and iron (Fe) concentrations in the plants were determined using an atomic absorption spectrophotometer, and phosphorus (P) content was determined by the molybdivanado-phosphoric acid method (Kitson and Mellon 1944) after wet ashing with sulfuric acid and hydrogen peroxide. Silica (SiO₂) content was estimated as an acid detergent insoluble ash (Goering and Van Soest 1970).

We sampled the *M. sinensis* leaves from strip A-1 almost every month for 1 year beginning in October 2004 and determined their Na, Mg, P, K, Ca, Fe, and SiO₂ content in dry matter by the same method used for analyses of nest materials. We were unable to determine dry matter weight of nest materials because they were partially carbonized during the fire. Therefore, we calculated changes in the ratios of either Ca or SiO₂ to the other 5 minerals, as both these minerals are more reliable for estimating nesting days due to their lack of mobility in the plant body. We then selected an appropriate mineral ratio that displayed either a steady increase or decrease throughout the year after spring. The dynamic of the selected ratio was fitted to the curves by the least-squares method, and the curves with the highest coefficients of determination were considered as candidates for deriving the equation used to estimate the nesting time. We also calculated the mean estimated error (days) for the candidate curves.

From strip A-1, 3 aerial nests were retrieved on 11 November 2004 and 1 bottom nest on 25 October 2005 (hereafter referred to as “testing nests”). The testing nests were treated in the same way as the bottom nests that were sampled after the spring fire, and nesting days for each were calculated using the equation derived from the candidate curves. We examined whether the nesting day could be calculated within the mean estimated error, taking into consideration the day the nests were observed. If not, it would prove that the candidate curve had led to the estimation of incorrect nesting time. Candidate curves were also used to calculate nesting times for those among the 20 bottom nests that contained florets of *M. sinensis*, and these were sampled after the fires in 2004 and 2005. We rejected candidate curves with which the nesting days were estimated to fall between April and August because florets of this plant are not formed before September. Nesting days of the 20 bottom nests were finally calculated using a curve that met these requirements.

Results

In 2004, we located 177.6 bottom nests ha^{-1} and 138.4 nests ha^{-1} in strips A-1 and C, respectively; in 2005, we located 56.6, 124.6, 63.1, and 58.1 nests ha^{-1} in strips A-1, A-2, B, and C, respectively. Bottom nests of the harvest mouse were distributed over all the strips investigated, and nest distribution showed no significant correlation with height, inclination, or direction of the slope in the strips analyzed ($P > 0.05$, Fig. 2). After burning, we found one charred frog body, a partly burned bird nest with an unhatched egg, and some larvae bodies but no mammal carcasses.

At Taketa, Oita AMeDAs Station, we had a summed precipitation of 35 mm, a summed sunshine duration of 64.6 h, and a mean wind speed of 3.7 m during the 10 days before the burning in 2004, and 12 mm, 72.7 h, and 4.9 m, respectively, in 2005. There were 7 days (61 cm maximum depth) of snow at Asosan Station in January in 2004 and 13 (43 cm maximum depth) in February 2005 (Table 1).

Nest P and K contents consistently decreased, whereas SiO_2 content continuously increased after spring emergence (% dry wt; Fig. 3). The SiO_2/Mg ratio steadily increased and produced the highest coefficient of determination of all mineral ratios (Fig. 4; Table 2). By using the SiO_2/Mg ratio curve, nesting days of the 3 testing aerial nests were calculated as 14 October, 3 November, and 28

October. These dates fell before the day on which the nests were first observed. The testing bottom nest was estimated to be built on November 25, which is 31 days after we observed it. The mean estimated error of this curve was 32.3 (days), and thus, the estimated nesting day was within the mean estimated error after the observation day. Of the 20 bottom nests sampled after the fires, 9 contained florets of *M. sinensis*, which not only covered the interior surface but were also part of the exterior walls and served as the framework of the nests. This confirmed that these nests were constructed after the flowering of *M. sinensis*. Using the SiO_2/Mg ratio curve, nesting times of nests that contained florets were estimated to fall between 21 October and 11 February, showing that the nests were constructed between September and March even after considering the mean estimated error. We therefore regarded this curve as the acceptable equation for estimating nesting time. Bottom nesting times were calculated to be between 3 October and 11 February or, more specifically, between early September and mid March, taking the mean estimated error into consideration.

The nest distribution was significantly biased toward the pixels dominated by *P. chino* in 2004 ($P = 0.013$) and pixels dominated by *M. sinensis* in 2005 ($P = 0.003$, Fig. 2). No nest was present in 43% of the 149 pixels dominated by *M. sinensis* and 30.4% of the 69 pixels

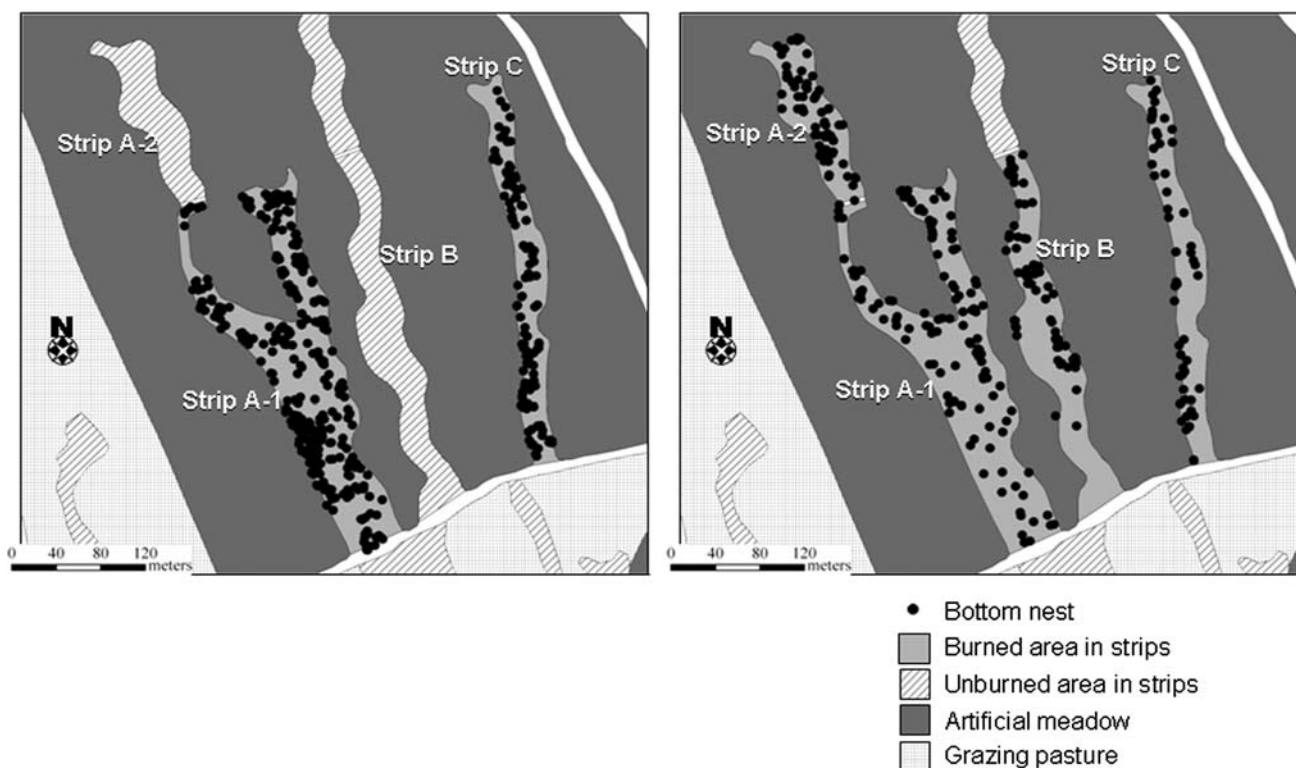


Fig. 2 Bottom-nest distribution observed in 2004 (left) and 2005 (right). Nests were distributed over the entire areas examined in this study

Table 1 The number of snowy days and maximum snow depth (cm) in each month at Asosan Meteorological Station 2003–2004 and 2004–2005

	December 2003–March 2004		December 2004–March 2005	
	NSD	MSD	NSD	MSD
December	15	1	10	8
January	7	61	26	16
February	7	37	13	43
March	9	12	17	14

NSD number of snowy days, MSD maximum snow depth (cm)

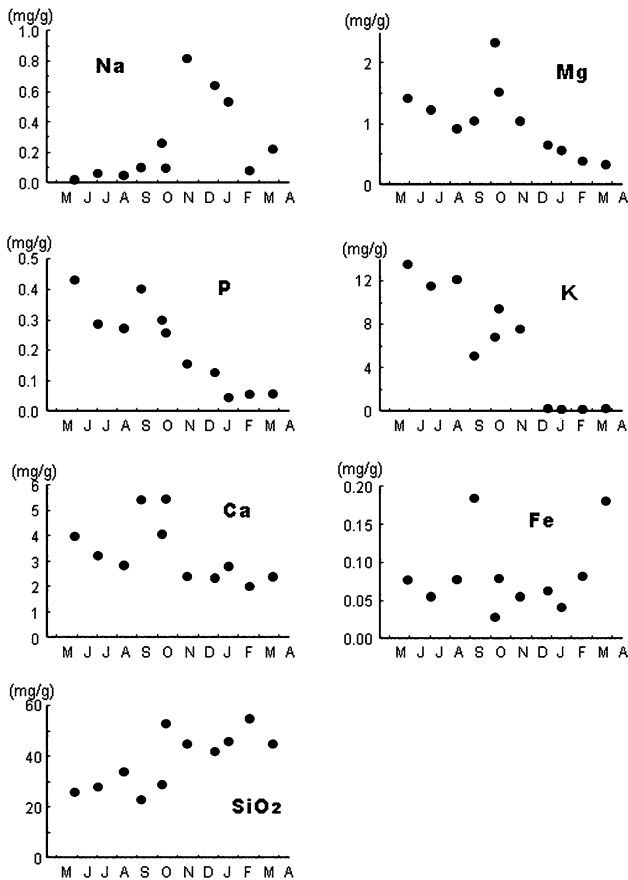


Fig. 3 Changes in sodium (Na), magnesium (Mg), phosphate (P), potassium (K), calcium (Ca), iron (Fe), and silica (SiO₂) contents in *Miscanthus sinensis* leaves from April to March in Kyushu, Japan

dominated by *P. chino* in 2004, and in 46.2% of the 234 pixels dominated by *M. sinensis* and 46.7% of the 90 pixels dominated by *P. chino* in 2005. In the pixels containing nests, 3.4 ± 2.4 [mean \pm standard deviation (SD)] and 3.7 ± 3.0 nests per pixel were observed for *M. sinensis* and *P. chino*, respectively, in 2004. In 2005, 1.8 ± 1.3 and 1.3 ± 0.6 nests per pixel were observed in *M. sinensis* and *P. chino*, respectively. Although trees occupied 42.6% of the area investigated in strip B, 63 nests were located in

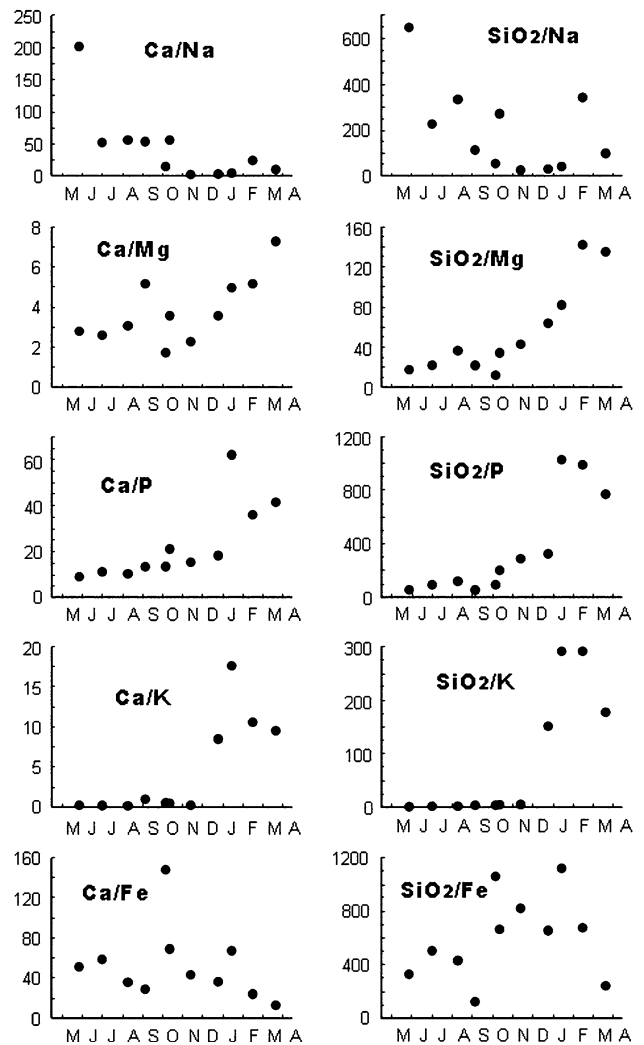


Fig. 4 Changes in the ratios of calcium (Ca) and silica (SiO₂) contents to contents of other minerals sodium (Na), magnesium (Mg), phosphate (P), potassium (K), calcium (Ca), and iron (Fe) in *Miscanthus sinensis* leaves from April to March in Kyushu, Japan

pixels dominated by grasses in contrast to only 2 under trees, and a significant bias toward grasses was detected ($P < 0.0001$, Fig. 2).

Discussion

This is the first study conducted on a large number of nests built on the ground by the harvest mouse, which has been very well known to build aerial nests in grass stalks. The wide distribution of bottom nests over the strips investigated in 2 consecutive years suggests that the harvest mouse routinely constructs this type of nest. The fact that the same type of nests have been identified in other places, including 3 different agricultural grasslands in the area and even in grasslands in Sambe, which is 269 km away from

Table 2 Candidate curves for the equation for estimating nesting time

Mineral ratios	Equations	R^2
Ca/Mg	$Y = (7.252 \times 10^{-7})X^3 - (3.282 \times 10^{-4})X^2 + (4.609 \times 10^{-2})X + 1.255$	0.7065
Ca/P	$\text{Ln } Y = (5.730 \times 10^{-3})X + 1.847$	0.7613
SiO ₂ /Mg	$Y = (1.054 \times 10^{-6})X^3 + (1.659 \times 10^{-3})X^2 - (3.458 \times 10^{-1})X + 3.537 \times 10$	0.9152
SiO ₂ /P	$\text{Ln } Y = (1.034 \times 10^{-2})X - 3.482$	0.8247

Each equation is a curve fitted for its mineral ratio dynamics with the highest coefficient of determination by using the least-squares method. *Ca* calcium, *Mg* magnesium, *P* phosphate, *SiO₂* silica, *Y* mineral ratio in the leaves of *Miscanthus sinensis*, *X* number of days after burning

Kuju (R. Ishiwaka, personal observation), supports this hypothesis. Our findings demonstrate that many individuals overwinter in bottom nests. Researchers may have overlooked some habitats of this species by exclusively interpreting data regarding the presence or absence of aerial nests (Trout 1978b; Surmacki et al. 2005; Kuroe et al. 2007). The adoption of such a method will lead to formulation of inadequate conservation policies regarding management of areas in which the harvest mouse overwinters.

Mineral content of *M. sinensis* leaves in the study area exhibited dynamics characteristic of subtropical graminous species. P and K contents in the leaves decrease as the plants grow. In advance of the flowering period in September, these minerals are translocated to the top of the plant and thereafter to seeds and roots for overwintering. K is much more likely to be mobile in the plant body and to get leached away by rainwater than the other minerals. The absence of K in the samples collected after November reflects complete loss due to translocation and leaching from the dead leaves during winter. P, K, and Ca contents were relatively high in September or October or both, probably as a result of fertilization of the adjacent artificial meadow. This indicates that the mineral dynamics in plant bodies can vary among different places and should be analyzed in each area when this method is applied in other studies. All minerals selected for the investigation were appropriate for the application of this method, showing high content values with small error margins compared with the other minerals. Analysis of SiO₂ is especially advantageous because of its high concentration and almost complete lack of mobility in the plant body; thus, SiO₂ concentrations are not affected by rainwater, even in leaves that have withered. This mineral constantly accumulates in the leaves after spring emergence, leading to clear and constant changes in the ratio of the concentrations of this mineral and that of others, which are appropriate for estimating nesting time.

The drastic decline in nest density from 2004 to 2005 can be explained by a difference in weather conditions before controlled burning. Heavy rainfall occurred before the fire in 2004, whereas fine weather prevailed for several days before the controlled burning in 2005; the drier conditions in 2005 probably led to complete combustion and

thus disappearance of a greater number of nests. Densities obtained in 2004 are therefore more reliable. Climate can also be used to explain the significant bias of bottom nests toward the patch of *P. chino* in 2004, which was not observed in 2005. The decrease in nests from 2004 to 2005 observed in the *P. chino* patch substantially contributed to the reduction in the total number of nests. The preference for the *P. chino* patch in 2004 might have been due to the heavy snowfall in the winter of that year, which compressed the vegetation into a thick layer of leaf litters, and many bottom nests were found embedded in this layer. It remains unclear whether the harvest mouse preferred the thick litter layer in the *P. chino* patch or the *P. chino* patch itself. Controlled burning in this patch without layer structure in drier conditions might result in the disappearance of a greater number of nests. The biased nest distribution in the *M. sinensis* patch in 2005 may be due to a smaller number of nests that were burnt away in the *M. sinensis* patch or a more regular use of this grass species for nest building on the ground, resulting from a substantial reduction in the number of nests located in *P. chino* patches. Topography of the strips also influences nest-site selection through the water system, wind direction, access to sunlight, and locations in which dead leaves accumulate. Although no significant correlation was observed between nest distribution and the inclination or direction of the slope using a geometric resolution of 15 m, some correlation might be found using higher resolutions.

The relationship between densities of aerial nests and number of harvest mice has not been studied in detail (Trout 1978b; Harris 1979). During our observation on some bottom nests, far more feces were observed in the nests than usually seen in aerial nests. The accumulated feces suggest continued use of the nests, nest sharing (i.e. huddling in a single nest), or both. Factors that have effects on the bottom nest density, therefore, would be as follows: (1) individual density, (2) possible nest sharing, (3) low likelihood of crumbling, leading to the longer duration of use of each nest, and (4) construction by not only breeding females but also males and nonbreeding females in order to avoid the cold. Further, we should consider the possible disappearance of some nests during burning and the use of underground passages as nests. Further research is required

on the relationship between densities of individuals and bottom nests as well as for aerial nests.

Chew et al. (1959) reported that counting carcasses after a fire is an easy method of obtaining quantitative data on population densities. The fact no mammal carcasses were detected during our survey suggests that harvest mice were not killed by the fire. There have been many conflicting views on the survival of rodents during fires. Mortality in some small mammalian species during burning has been reported (Chew et al. 1959; Erwin and Stasiak 1979). However, many scientists suggest that most individuals are not killed by fires (Beck and Vogl 1972; Vogl 1973; Clark and Kaufman 1990). In general, the effects of fire on burrowers are indirect (Howard et al. 1959; Geluso et al. 1986; Simon 1991) because the temperature underground would not significantly increase during burning (Lawrence 1966). We observed that although harvest mice cannot dig, captive harvest mice used an artificial underground burrow, i.e., a tube sunk into the soil (R. Ishiwaka, personal observation). In captivity, this species bred in an underground nest built in compost of shredded *Phalaris* sp. and soil (Banks 1973). In our study area, the large Japanese field mouse *Apodemus speciosus*, the small Japanese field mouse *A. argenteus*, and the Japanese field vole *Microtus montebelli* shared this habitat with the harvest mouse (R. Ishiwaka, personal observation). Many burrows were observed in each strip, and harvest mice could easily use these burrows if necessary. We confirmed that some bottom nests were connected to underground burrows, and nest materials were laid on the lower and vertical surfaces from the entrance to the back of the cavity for a length of approximately 20 cm. These factors would allow the harvest mouse to escape from fires and survive burning. Many bottom nests in the habitat burned every year at least prove that the population is not damaged by this controlled fire, even if some harvest mice are killed.

Although high densities of harvest mice have often been reported from habitats that have been managed to a certain extent (Harris 1979, 1991; Haberl and Krystufek 2003; Surmacki et al. 2005), the optimal frequency of disturbances in these habitats has not yet been determined. Despite the relatively high density of nests in strip B in 2005, all nests were detected in grasses except for two. Unless any disturbances occur regularly, grasslands in Japan will rapidly shift to forests because of the heavy rainfall. Therefore, many trees were present in strip B, which had been more or less abandoned since the last burning more than 10 years ago. Controlled burning is considerably effective in conserving the habitat of the harvest mouse, although annual management does not seem to be necessary, suggested as well in a study on aerial nests (Surmacki et al. 2005). Farmers have conducted controlled burning in the Aso and Kujū area for more than

1000 years in order to grow and harvest *M. sinensis* as a roof-building material, livestock feed, and green manure for crop fields. This agricultural practice has maintained the grassland landscape in which many rare herbaceous species and grass-dwelling animals have survived.

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