Different Patterns of Food Consumption and Locomotor Activity among Taiwanese Native Rodents, Formosan Wood Mice (*Apodemus semotus*), and Common Laboratory Mice, C57BL/6 (*Mus musculus*)

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Abstract

The comparisons of food consumption and locomotor activity among Taiwan native rodents, Formosan wood mice (*Apodemus semotus*), and laboratory mice, C57BL/6, were examined in this study. The food consumption exhibited the circadian rhythmicity, *e.g.* higher in the lights-off period and lower in the lights-on period, in either Formosan wood mice (WM) or C57BL/6 mice. We also found that Formosan WM ate more food than C57BL/6 mice in the lights-off period and the whole day in males, but not in females. Similarly, the male Formosan WMs had more locomotor activities than the male C57BL/6 mice in the lights-off period, but this phenomenon did not appear in female mice. These results indicated that even though the Formosan WMs have been successfully inbred in the laboratory, they still keep more native paradigm than the laboratory C57BL/6 mice do. This study is the first report to provide basic physiological comparisons on native and common laboratory mice.

Key Words: circadian rhythm, food intake, distance of movement, Formosan wood mice

Induction

Taiwan is a mountainous island with 2/3 of its area at elevation above 1000 m. The steep Central Mountain Range largely runs along the longitudinal axis of the island with the highest peak at nearly 4,000 m (1). A substantial change exists and creates a sharp ecological gradient. Kano (5) studied faunistic affinity and indicated that the vertebrate fauna in Taiwan consists of two major elements of different geographic origins: the Oriental element confined predominately in the lowlands and the Palaearctic element mostly in the highlands (7).

Thirteen species of murine and microtine rodents in Taiwan have been found, of which nine mainly distributed at elevation under 2,000 m, and the other 4 above 1,500 m (9, 10, 21, 22). Therefore, the distributions of these rodents in Taiwan can be divided into two regions: one is the high-elevation region, and the other is the low-elevation region. Lin (9) had proposed the elevation between 1,500 and 2,000 m to be an interface zone for mammals in Taiwan. The genus *Apodemus* comprises 13 species of wood mice (WM) distributing widely in the Palearctic region (3).

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Received: December 15, 2006; Revised (Final Version): July 17, 2007; Accepted: August 20, 2007.

The Formosan WMs (*Apodemus semotus*) is endemic to Taiwan and commonly found in the central and southern mountainous areas of the island (2). Population biology and postnatal growth of Formosan WMs have been studied (11, 13). Additionally, several reports documented that the seasonal reproductive pattern of wild Formosan WMs based mainly on the gravimetric and histological measurements of reproductive organs and plasma androgen levels (4, 8, 11). Recently, the collaborative teams from Tzu-Chi University and National Taiwan University successfully have inbred this wild Formosan WM in the laboratory environments. In the preliminary subjective observation, we found that the Formosan WMs exhibit nocturnal and hyper-locomotor activities, including jumping and searching behaviors. In fact, these laboratory-inbred Formosan WMs can jump above 50 cm height and escape from the 1 m height of platform to the ground as they live in the natural environments. Therefore, the major focus of this study is to investigate the basic physiological aspects from comparing the Formosan WMs with common laboratory mice by non-invasive methods. In this study, we focused on the observation of whether the Formosan WMs have with similar levels of food consumption and locomotor activities in the circadian status as the common laboratory-inbred mice, C57BL/6.

Materials and Methods

Four-month-old adult unmated male and female Formosan WMs (*Apodemus semotus*, n = 24), weighing 25-35 g, and laboratory C57BL/6 mice (n = 24), weighing 25-30 g, were purchased from the Laboratory Animal Center at Tzu Chi University (Hualien, Taiwan, ROC), and were housed in a temperature (22 ± 1 °C)- and light (lights on from 0600 to 1800)-controlled room with free access to rodent chow and tap water. The common inbreeding environments between the C57BL/6 mice and Formosan WMs were similar, except adding clovers into the common rodent bedding for Formosan WM use. For easy comparison of food consumption and locomotor activities in the laboratory environment, we used the Zeitgeber time (ZT), which is identified as the lights on, as the time index. The Formosan WMs were kept in the standard rat polypropylene cage (45 cm long × 30 cm wide × 30 cm high), while the Laboratory Animal Center at Tzu Chi University has successfully inbred them ever since several years ago. Therefore, to compare the differences between these mice, standard mouse (25 cm long × 15 cm wide × 15 cm high) and rat polypropylene cages were used for both types of mice during the tests, *i.e.*, the C57BL/6 mice were transferred from the standard mouse cage to the standard rat cage, and Formosan WMs were transferred to the standard mouse cage for tests. The mice were habituated to the housing conditions for at least 1 week before the experimental manipulations, and were treated without suffering in accordance with the guidelines of the Institutional Animal Care and Use Committee of Tzu Chi University.

Because the amount of food consumption in a single mouse situation is too small to assess, we put three mice in a single cage for measurement of food consumption. The food consumption of mice were manually measured every 2, 4, and 12 h. The body weight of each mouse was routinely measured at 30 min after lights-on every day. We also measured the water intake of mice in each cage during the whole day. To analyze the locomotor activities, we used a video-tracking software (EthoVision 3.0, Noldus Information Technology, Wageningen, Netherlands) to measure the distance of movements in mice. The video tracking system was equipped with a digital infrared sensitive CCD camera (A41I11N, Kera Electronics, Taipei, Taiwan, ROC), infrared illuminators (KE-101, Kera Electronics, Taipei, Taiwan, ROC), IBM-compatible PC, and standard rat and mouse plastic cages. In the lights-on situation, the infrared sensitive CCD camera directly detected the mice's movements by visible lights in their cages. In the lights-off situation, the infrared illuminators were automatically launched, and the mice's movements were still detectable by the infrared sensitive CCD camera. First, the EthoVision software was used to identify the mice's shapes by the relative contrast. After the computer calculated the mass center of the mice, the cursors would appear on the screen, positioned by the two axis coordinates (X_1, Y_1). Then, the software would automatically calculate the distance that the cursors moved from (X_1, Y_1) to (X_2, Y_2). This method had been described in a previous study (20).

Experimental Designs

Every three Formosan WMs or C57BL/6 laboratory mice were randomly kept in standard rat cages for one-week habituation, and then we measured manually the food intake every two hours for two days. The mice were separated and kept in individual situation for another one-week habituation, and then their behaviors were recorded for two whole days. Similar procedures were repeated except using the standard mouse cage instead of the standard rat cage.

Statistical Analysis

The data were presented as mean ± SE. Differences between species groups (Formosan WM or C57BL/6 mice) with different time points of food consumption (from ZT 2 or ZT 4 to ZT24) were determined by a two-way ANOVA with species as the independent variable and time points as the other. Otherwise, the two-tailed unpaired Student's *t*-test was used to compare two different groups. If significant ($P < 0.05$), the ANOVA was followed by post-hoc Student-Newman-Keuls' multiple-range test which was used to test the significance of differences among groups.

Results

The body weights of male Formosan WMs and laboratory C57BL/6 mice were 29.87 ±1.36 and 28.94 ±0.78. The lengths of male Formosan WMs and laboratory C57BL/6 mice were 9.34 ± 0.42 and 9.12 ±0.36. In contrast, the body weights of female Formosan WMs and laboratory C57BL/6 mice were 24.38 ± 0.67 and 24.54 ± 0.32. The lengths of female Formosan WMs and laboratory C57BL/6 mice were 8.78 ± 0.51 and 8.34 ± 0.43. Therefore, the lengths and ages between two groups in the same sex exhibited no difference. The water consumptions of Formosan WMs and laboratory C57BL/6 mice during the whole day were 12.38 ± 3.51 and 11.96 ± 3.17, respectively. In addition, the water intake between Formosan WMs and laboratory C57BL/6 mice in the same sex made no difference either during the lights-on or lights-off periods (data not shown). The food intake per body weight as the food consumptive index was assessed on Formosan WMs and laboratory C57BL/6 mice. Firstly, both 2 h and 4 h of food consumption showed diurnal rhythm in either Formosan WMs (2 h, Student-Newman-Keuls' ANOVA: $F_{11,132} = 10.05$, $P < 0.001$; 4 h, ANOVA: $F_{5,66} = 18.61$, $P < 0.001$) or C57BL/6 mice (2 h, ANOVA: $F_{11,132} = 32.52$, $P < 0.001$; 4 h, ANOVA: $F_{5,66} = 47.13$, $P < 0.001$) (Fig. 1). In the comparison of food consumption on different species without distinguishing the gender, the laboratory C57BL/6 mice ate more food than the Formosan WMs either at ZT 8 (Unpaired *t* test: $t_{22} = 3.376$, $P < 0.01$), ZT 10 ($t_{22} = 2.490$, $P < 0.05$), and ZT 16 ($t_{22} = 2.625$, $P < 0.01$) in 2 h duration, or at ZT 8 in 4 h duration ($t_{22} = 2.502$, $P < 0.05$) (Fig. 1A,B). On the other hand, Formosan WMs ate more food than the laboratory C57BL/6 mice either at ZT 20 ($t_{22} = 2.191$, $P < 0.05$), ZT 22 ($t_{22} = 3.202$, $p < 0.01$), and ZT 24 ($t_{22} = 2.201$, $P < 0.05$) in 2 h duration, or at ZT22 ($t_{22} = 2.091$, $P < 0.05$) and ZT 24 ($t_{22} = 3.564$, $P < 0.01$) in 4 h duration (Fig. 1A,B). To study whether gender was the factor, we recalculated the food consumption by differentiating male and female groups. Male laboratory C57BL/6 mice consumed more food than male Formosan WMs only at ZT 8 in 2 h duration ($t_{22} = 2.196$, $P < 0.05$; Fig. 2A). In contrast, male Formosan WMs ate more food than male laboratory C57BL/6 mice, both at ZT 12 ($t_{22} = 2.500$, $P < 0.05$), ZT 18 ($t_{22} = 3.264$, $P < 0.01$), ZT 20 ($t_{22} = 2.507$, $P < 0.05$), ZT 22 ($t_{22} = 2.231$, $P < 0.05$), and ZT 24 ($t_{22} = 2.372$, $P < 0.05$) in 2 h duration and at ZT 20 ($t_{22} = 2.171$, $P < 0.05$), and ZT 22 ($t_{22} = 2.083$, $P < 0.05$) in 4 h duration (Fig. 2A,B). The trend of food consumption on female mice was different from that of the male mice. The female laboratory C57BL/6 mice had higher food consumption, either at ZT 8 ($t_{22} = 3.249$, $P < 0.01$), ZT 10 ($t_{22} = 4.091$, $P < 0.01$), ZT 16 ($t_{22} = 4.944$, $P < 0.01$) in 2 h duration, or at ZT 8 ($t_{22} = 2.421$, $P < 0.05$) and ZT 16 ($t_{22} = 4.562$, $P < 0.01$) in 4 h duration (Fig. 2C,D). On the other hand, female Formosan WMs ate more food both at ZT 22 ($t_{22} = 2.429$, $P < 0.05$), ZT 24 ($t_{22} = 2.346$, $P < 0.05$) in 2 h duration, and at ZT 24 ($t_{22} = 2.196$, $P < 0.05$) in 4 h duration (Fig. 2C,D).

Using the food intake per body weight during lights-on and -off periods, and the whole day as the indices, we found that Formosan WMs ate more than C57BL/6 mice during the whole day ($t_{22} = 2.114$, $P < 0.05$; Fig. 3A). Additionally, the total food consumption during the whole day of the male Formosan WMs was higher than that of the male C57BL/6 mice, but not of the female mice ($t_{22} = 2.132$, $P < 0.05$; Fig. 3A). We

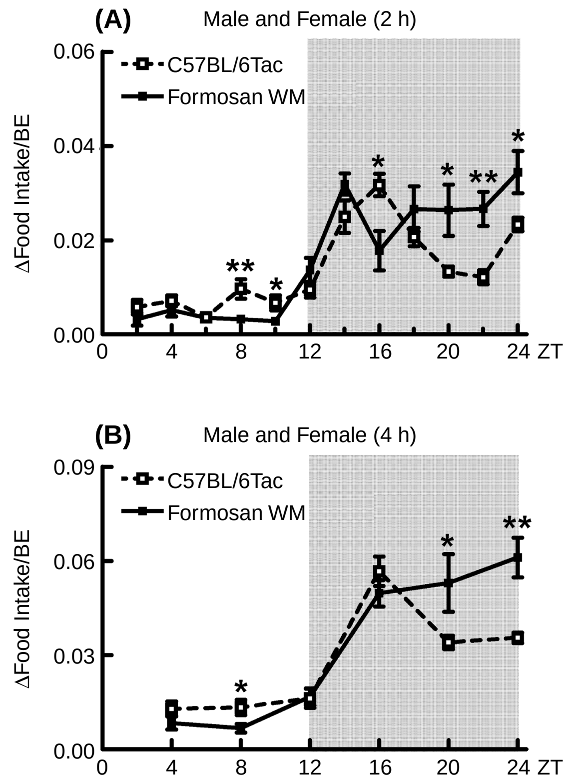

Fig. 1. Difference of food intake/BW in the laboratory C57BL/6 mice and Formosan WM manually measured by (A) every 2 h and (B) 4 h without distinguishing its gender. The shadow indicated the lights-off period. The vertical line above and below each dot represents the SE (n = 12). $^*P < 0.05$; $^{**}P < 0.01$ compared with the laboratory C57BL/6 mice in the same time point.

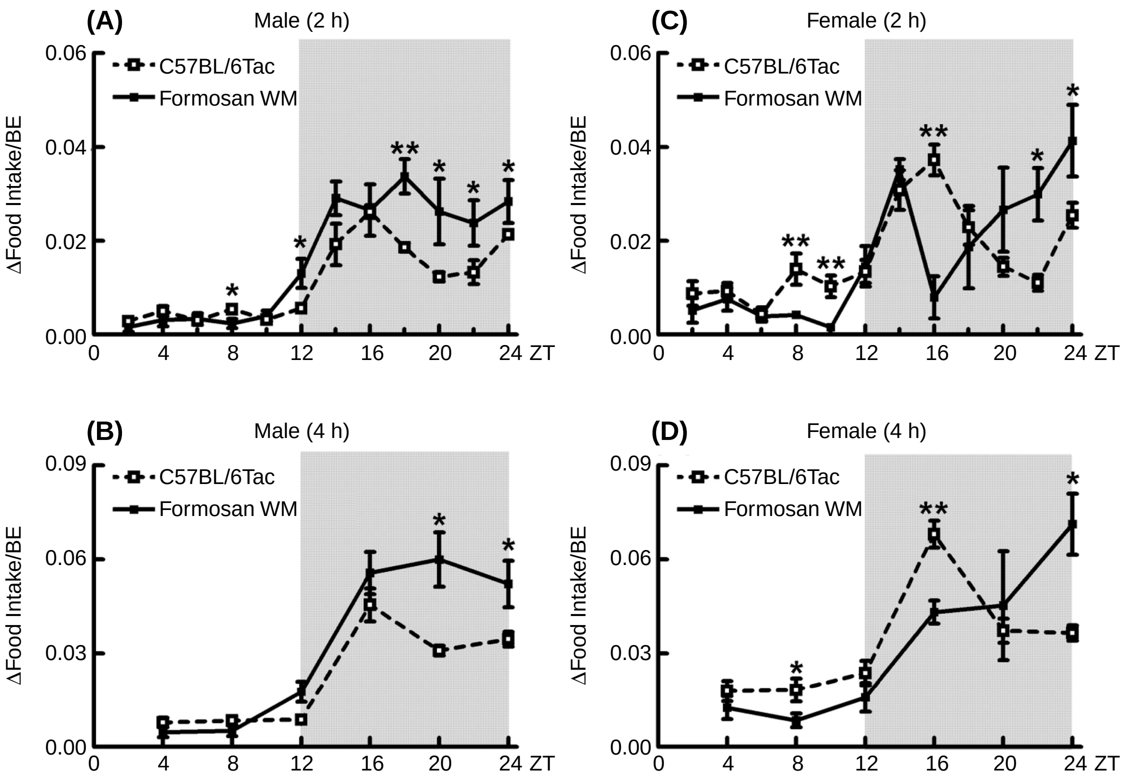

Fig. 2. Difference of food intake/BW in the laboratory C57BL/6 mice and Formosan WM manually measured by (A,C) every 2 h and (B,D) 4 h, distinguished by gender (Male: A,B; Female : C,D). The shadow indicated the lights-off period. The vertical line above and below each dot represents the SE (n = 6). *P < 0.05; **P < 0.01 compared with the same gender laboratory C57BL/6 mice in the same time point.

noticed that the food consumption in the lights-on period, the laboratory C57BL/6 mice and Formosan WMs did not make any difference (Fig. 3B). Conversely, male Formosan WMs ate more food in the lights-off period than male C57BL/6 mice, and female mice did not show this trend (t_{22} = 3.119, P < 0.01; Fig. 3C). Similarly, the total consumption in male and female C57BL/6 mice was lower than that of the Formosan WMs in the lights-off period (t_{22} = 3.201, P < 0.01; Fig. 3B). Using the video tracking system to monitor the distance of movement on Formosan WMs and C57BL/6 mice as the index of locomotor activities, either male Formosan WMs (Student-Newman-Keuls' ANOVA: $F_{11,132}$ = 35.31, P < 0.001) or male C57BL/6 mice (2 h, ANOVA: $F_{11,132}$ = 31.74, P < 0.001) showed diurnal rhythm (Fig. 4A). The locomotor activities of female Formosan WMs and C57BL/6 mice also revealed diurnal rhythmicity (data not shown). However, only male, but not female, mice had the rhythmic difference between species. Male C57BL/6 mice and Formosan WMs in the lights-on period had higher activities at ZT 2 (Unpaired t test: t_{22} = 2.314, P < 0.05; Fig. 4A) and ZT 6 (t_{22} = 2.638, P < 0.01; Fig. 4A), respectively. In the lights-off period, the overall locomotor activities of male Formosan WMs were more than those of the male C57BL/6 mice ($t_{22\ Min}$= 2.184; $t_{22\ Max}$= 2.472, P < 0.05 and 0.01; Fig. 4A), especially at ZT 20 and ZT 22 (t_{22} = 4.372 and 4.987, P < 0.01; Fig. 4A). However, there was no difference in the lights-on period between male Formosan WM and C57BL/6 mice on the total distance of movement (Fig. 4B middle panel). Male Formosan WMs had more locomotor activities than male C57BL/6 mice during the lights-off (t_{22} = 2.358, P < 0.05; Fig. 4B right panel) and 24-h (t_{22} = 2.476, P < 0.05; Fig. 4B left panel) periods. Additionally, the locomotor activities of female Formosan WMs and C57BL/6 mice were similar in either lights-on or lights-off periods (data not shown).

Discussion

Formosan WMs (*Apodemus semotus*) is a unique type of Taiwanese rodents and only very few reports had studied this species before. Most of the studies on Formosan WMs are related to ecological or reproductive issues (4, 8, 11-13), but not on their physiological activities. Because the Laboratory Animal Center at Tzu Chi University have successfully inbred Formosan WM in the common laboratory environment, we found that Formosan WMs had special behaviors from our

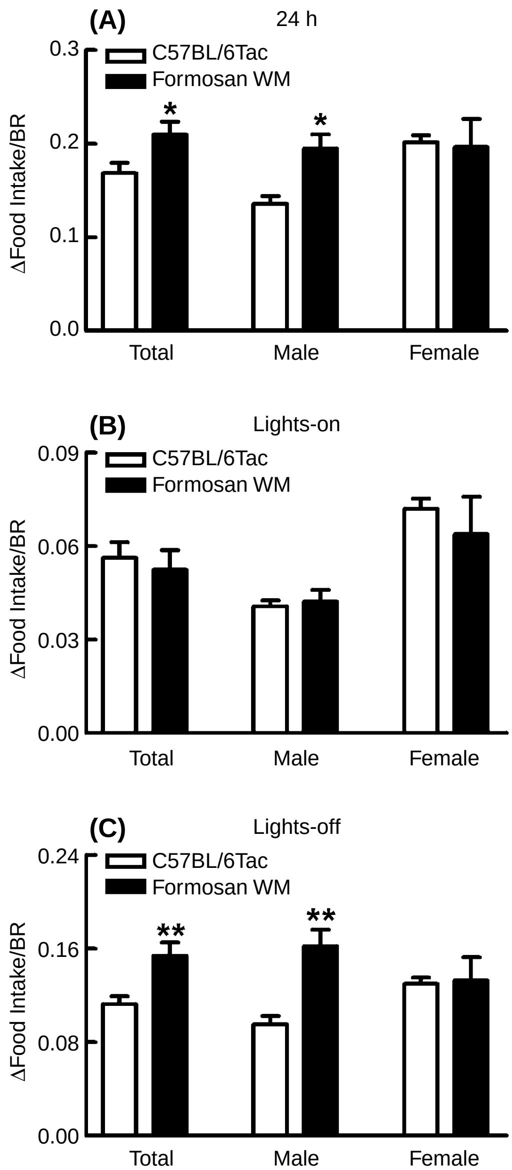

Fig. 3. Difference of food intake/BW in the laboratory C57BL/6 mice and Formosan WM in (A) the whole day, (B) the lights-on period, and (C) the lights-off period. The vertical line above each bar represents the SE (n = 6-12). *P < 0.05; **P < 0.01 compared with the laboratory C57BL/6 mice in the same period.

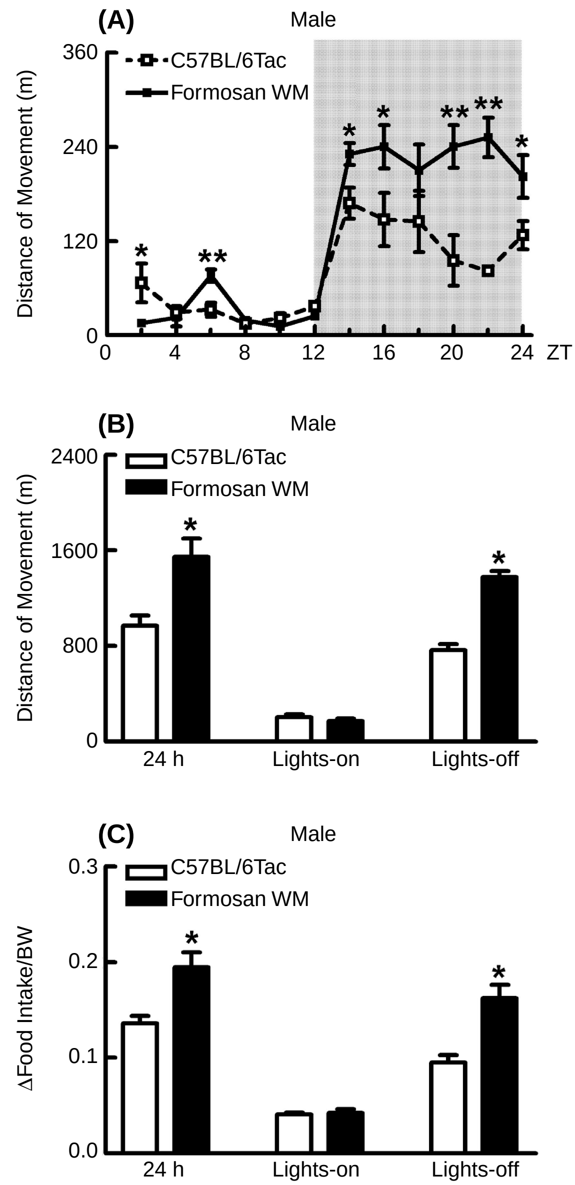

Fig. 4. Difference of locomotor activity in the male laboratory C57BL/6 mice and male Formosan WM in (A) every 2 h and (B) the lights-on, -off or 24-h periods. The panel C was adapted from the male part of Fig. 3A-C. The shadow indicated the lights-off period. The vertical line represents the SE (n = 6). *P < 0.05; **P < 0.01 compared with the laboratory C57BL/6 mice in the same time point or period.

previous subjective observations, such as hyper-locomotor activity and jumping ability. This report is the first study ever that focuses on the basic physiological comparison of Formosan WMs and common laboratory C57BL/6 mice. The following findings in the present study were obtained. First, food consumption of Formosan WMs and C57BL/6 mice exhibited circadian rhythmicity, either at two-h or at four-h intervals. Second, during the lights-off period and the whole day, the food consumption of Formosan WMs was higher than that of the C57BL/6 mice. Third, this phenomenon was mainly found in the male Formosan WMs, but not in the females. Finally, male Formosan WM also had higher locomotor activities than male C57BL/6 mice. All these findings indicated that Formosan WM differed from laboratory C57BL/6 mice at least in male mice.

Overall, the nocturnal rodents showed higher food consumption in the lights-off period than in the lights-on period. Formosan WMs are similar to laboratory C57BL/6 mice, either in rhythmic patterns of behaviors of food consumption or locomotor activity. Both food consumption and locomotor activities exhibited the circadian rhythmicity, *i.e.*, higher rhythm in lights-off period and lower rhythm in lights-on periods. These data showed that Formosan WMs had adapted to the laboratory environment and did not lose their natural behaviors after 2-year-inbreeding. However, Formosan WMs still revealed differences from common laboratory C57BL/6 mice. The major difference was that Formosan WMs exhibited higher food consumption and distance of movement than laboratory C57BL/6 mice in the lights-off periods, especially in male but not in female. Moreover, these two parameters showed a good correlation (Please see Fig. 4, B and C). We tried to identify the causes by inferences. One explanation was that male Formosan WMs had higher distance of movement because they needed to approach food more. However, the video tracking path from our data did not find this (data not shown). Therefore, the other possibility was that male Formosan WMs ate more food because they moved farther. Male Formosan WMs spent more time in fighting or playing around in the lights-off periods while we manually measured the food consumption from our observations in this study. Due to the energy-intensive activities, male Formosan WMs needed to eat more and to compensate for energy lost. However, we cannot conclude whether this finding is natural or not. Male Formosan WMs fought or played more in the laboratory environment despite the territory or space insufficiency. In natural fields, male Formosan WM or Formosan WM families might need more space to be separated. We had to make an important point that Formosan WMs need more space to breed offspring, so the common standard mouse cage cannot be used for breeding Formosan WM. Mating behavior cannot be successful or offspring would be terminated by the infanticidal behavior in standard mouse cages. Previous studies have shown that the infanticidal behavior was involved in multiple factors, such as hormonal and experimental factors (16-18). In fact, the genetic factor cannot be ruled out (6, 14, 15, 19). This infanticidal behavior was improved by breeding Formosan WM in common standard rat cages. The spatial factor may play a critical role on the infanticidal behavior in Formosan WMs. We measured the individual locomotor activity in this study, nevertheless, the space factor still cannot be ruled out. At least, male Formosan WMs moved around and/or explored a lot than laboratory C57BL/6 mice during the lights-off period. Additionally, male Formosan wood mice also showed higher response than laboratory C57BL/6 mice during the behavioral tests, such as the novel environment and elevated plus maze (data not shown). Male Formosan wood mice might be useful as the mice model of spontaneous attention deficit hyperactivity disorder (ADHD) or high anxiety. Further studies are needed to address these issues.

In conclusion, this is the first report indicating the basic physiological indices on successful inbreeding Formosan WM in common laboratory environment. The different behaviors of Formosan WMs compared to those of the laboratory C57BL/6 mice are distinctive and reproducible. We hope this study can induce further interesting studies on these Taiwanese unique rodents, Formosan WMs (*Apodemus semotus*).

Acknowledgments

We are grateful for the technical assistance from CY Lee, RJ Chen, LH Wu and the Laboratory Animal Center in Tzu Chi University, and we thank the helpful comments provided by Professor M. H. Wang. This study was supported in part by National Science Council of Taiwan grants (NSC93-2320-B-320-010 and NSC94-2320-B-320-006) to Kun-Ruey Shieh and (NSC94-2320-B-277-001) to Shu-Chuan Yang, a Tzu Chi University Research Grant (TCMRC9314A) to Kun-Ruey Shieh, and a Tzu Chi College of Technology Research grant (TCCT931A04) to Shu-Chuan Yang.

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