J. Agric. Sci., Tokyo Univ. Agric., 54 (1), 15-19 (2009)
東京農大農学集報, 54 (1), 15-19 (2009)

Relationship between the Season of Falling into Side Gutter and the Breeding Season of the Large Japanese Field Mouse *Apodemus speciosus*

By

Ken-ichirou TAKANAKA*, Motokazu ANDO*, Takashi AMANO* and Hiroshi OGAWA*

(Received November 18, 2008/Accepted January 23, 2009)

Summary : Mean testicular size and plasma testosterone concentration in matured male the Large Japanese Field Mouse, *Apodemus speciosus,* were measured in an effort to determine if a relationship exists between the breeding season and what has been termed the falling season into roadside gutter. The mice were captured from December 2003 to November 2004 in Fujinomiya, Shizuoka, Japan. In total, thirty-five matured male mice were studied. The mean value of testicular length was the high level from February to September. During these months the testicular length was more than 12 mm. In contrast the mean testicular weight and plasma testosterone concentration peaked in April and August. The mean plasma testosterone concentration decreased to basal levels in June. The breeding season of the Large Japanese Field Mouse is considered to occur twice in the spring and autumn in the area under study. Despite the fact that the male Large Japanese Field Mouse was observed to be in a condition assumed to be morphologically agreeable to reproductive activity, it was clear that the mouse was not in an endocrinologically reproductive state. In addition, our data indicated that testicular weight correlated better with testosterone levels than did testicular length. We consider testicular weight could be a better indicator of reproductive activity than testicular length. Therefore, we considered that the falling season into side gutter was related to the breeding season activity.

Key words : Apodemus speciosus, fall into side gutter, breeding season, testicular size, testosterone

Introduction

The fact that small animals fall into roadside gutters and die because they are unable to escape has become a recent issue of concern. We determined that the average frequency of this occurrence exceeded one animal per day, and recognized that there was a seasonality to this roadside gutter falling statistic[1]. We hypothesized that the falling season into side gutter was related to the breeding season of fallen animals[1].

The Large Japanese Field Mouse is distributed throughout Japan[2-4] and provides bait for raptors such as owls[5-8]. The mouse also functions to distribute seeds throughout the forests of Japan[9-13]. It has been reported that the breeding season of the mice occurs once in the summer[14,15] and winter[16,17] and twice in spring and autumn[18-25] of each year. TAKANAKA *et al.* observed many fallen individuals of the Large Japanese Field Mouse *Apodemus speciosus*[1].

In the present study, testicular length, weight and plasma testosterone concentration of matured male individuals of the Large Japanese Field Mouse were measured to examine the relationship between the breeding season and the falling season into side gutter of these animals.

Materials and Methods

Animals

The study area was Fujinomiya, Shizuoka, Japan (35°24′N, 138°34′E, altitude 810-860 m). We previously investigated the side gutter falling patterns of mam-

* Department of Animal Science, Graduate School of Agriculture, Tokyo University of Agriculture

mals in this area[1]. All Large Japanese Field Mouse specimens were captured using a Sharman trap and were collected five days in each month from December 2003 to November 2004. The mice were kept in individual cages using their natural photoperiod and habitat temperature until they were examined for gender and maturity. We studied thirty-five matured male mice in our work. Male mice weighing more than 28 g were divided by body weight, according to MURAKAMI[22]. Ethical considerations to the animals were in compliance with the Standards Relating to the Care and Management of Experimental Animals.

Plasma testosterone determination

Blood sampling was performed from 12 : 00 to 13 : 00 pm in order to control for diurnal fluctuations in hormone levels. Blood samples were collected from the heart using a heparinized syringe under Nembutal anesthesia. The plasma samples were separated by centrifugation at 1,600 g for 10 minutes at 4℃ and then stored at −80℃ until they were assayed for testosterone. The plasma testosterone concentration of each sample was determined using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI), according to the manufacturer's protocol. The intra assay coefficient was 8%.

Testes measurements

The body measurements were performed and the testicular descent was observed. The testes were removed from mice after euthanization by an overdose of anesthetic. The testicular length and weight were measured using a caliper and an electronic balance, respectively. The mean values of these measurements were calculated for the right and left testes. The reproductive activity of the mice was confirmed upon examination of the white tubule in the caudal epididymis according to JAMESON[26].

Statistical Analysis

To compare the values for each month, the testicular length, weight, and plasma testosterone concentration were analyzed by a one-way ANOVA[27,28]. Three samples were used for each month except for January when n=2. Duncan's new multiple range test[29,30] was used to separate each mean. The coefficients of correlation were calculated for the annual changes in plasma testosterone concentration, testicular length and weight. These data were shown as the mean±SE of the three samples.

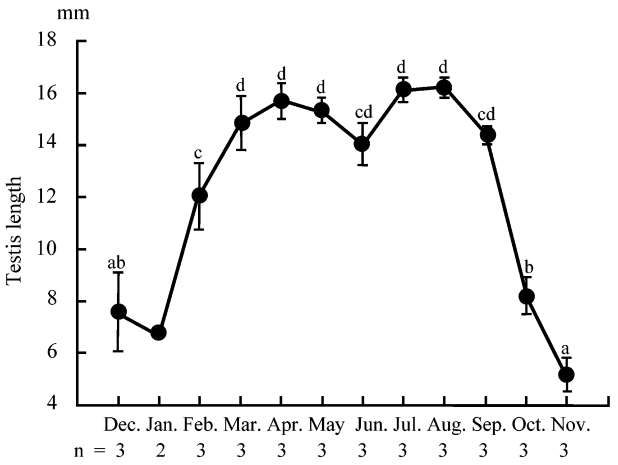

Fig. 1 Annual changes in the mean testicular length of Large Japanese Field Mouse. Each point represents the mean of three mice except in January (n=2), and the vertical bars represents SE. Points with different letters are statistically significant by Duncan's new multiple range test ($p<0.05$)

Results

The mean values measured for the testicular length were significantly higher from February to September than the values collected from October to January ($p<0.05$) (Fig. 1). These data indicated that the maximum length (16.2±0.4 mm) occurred in August and the minimum length (5.1±0.6 mm) occurred in November. While the values were less than 8.1 mm from October to January, they were 12 mm or more from February to September. These increased 1.8 times from January (6.6 mm) to February (12±1.3 mm) and decreased significantly, by approximately half, from September (14.3 ±0.3 mm) to October (8.1±0.7 mm) ($p<0.05$). Testicular descent and the white tubule in the caudal epididymis were observed from February to September, but not from October to January. The coefficient of correlation was $r=0.723$ between the annual changes in the mean values for testicular length and plasma testosterone concentration ($p<0.05$).

The mean value for the testicular weight showed two peaks, in April and August. The maximum value (0.681 ±0.034 g) occurred in August and the minimum value (0.019±0.007 g) was measured in November ($p<0.05$) (Fig. 2). This measurement increased from January (0.053 g) to April (0.661±0.078 g), decreased in June (0.463 ±0.075 g) and increased again until August ($p<0.05$). It remained less than 0.053 g from October to January ($p<0.05$). The coefficient of correlation was $r=0.777$ between the annual changes in the mean values ob-

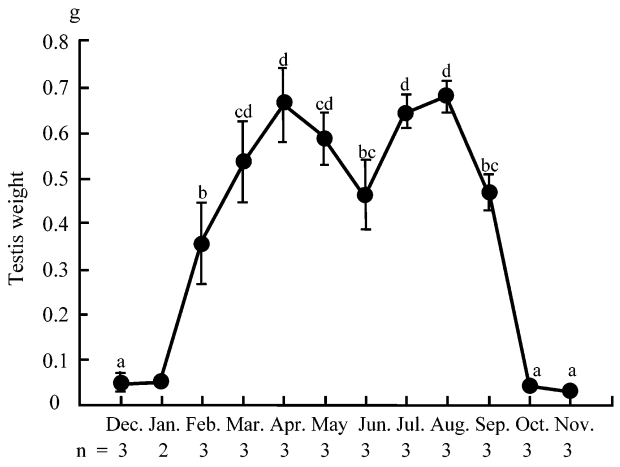

Fig. 2 Annual changes in the mean testicular weight of Large Japanese Field Mouse. Each point represents the mean of three mice except in January (n=2), and the vertical bars represents SE. Points with different letters are statistically significant by Duncan's new multiple range test ($p<0.05$)

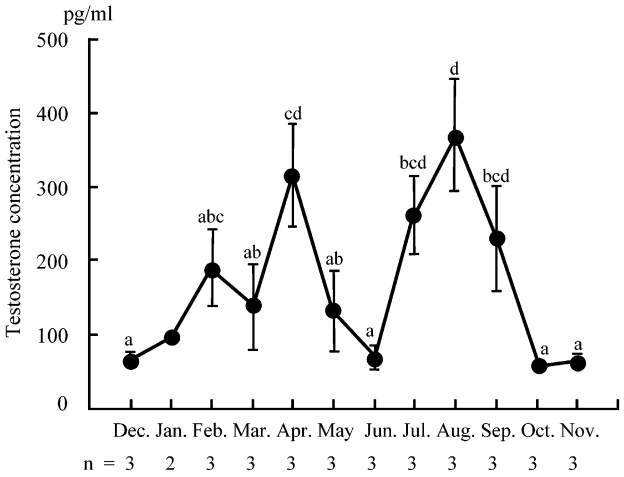

Fig. 3 Annual changes in the mean testosterone concentration of Large Japanese Field Mouse. Each point represents the mean of three mice except in January (n=2), and the vertical bars represents SE. Points with different letters are statistically significant by Duncan's new multiple range test ($p<0.05$)

tained for the testicular weight and plasma testosterone concentration ($p<0.05$).

The mean value of the plasma testosterone concen tration peaked twice, in April and in August. The maximum value (370±74.6 pg/m*l*) was measured in August and the minimum value (55.8±4.4 pg/m*l*) occurred in October ($p<0.05$) (Fig. 3). It increased 2.3 times from March (139.4±59.5 pg/m*l*) to April (315.5±70.4 pg/m*l*), decreased until June (70.2±15.3 pg/m*l*) and increased again until August ($p<0.05$). The values of the plasma testosterone concentration were significantly low (less than 100 pg/m*l*) in June and from October to January ($p<0.05$).

Discussion

The Large Japanese Field Mouse had the greatest testicular length from February to October at Yatsugatake in Honshu[14]. MURAKAMI[22] reported that testicular length at sexual maturation for individuals of this mouse species was more than 12 mm. We observed that the testicular length of individuals was more than 12 mm from February to September in the present study. Therefore, the breeding season of the male Large Japanese Field Mouse seemed to occur from February to September in the study area.

However, the mean values measured for the testicular weight and plasma testosterone concentration had two peaks ($p<0.05$), in April and August (Fig. 2, Fig. 3). The plasma testosterone concentration decreased to a basal level in June. In previous studies, the breeding season of this mouse was found to occur twice in the spring and autumn in Hiroshima[18,21], Niigata[19,20], Kyoto[22], Nagoya[23], Saitama[24], and Nara[25]. The reported annual changes in the mean values for testicular size correlated with our present study.

Testicular development is regulated by gonadotropin and spermatogenesis requires a high level of testosterone in mammals[31]. But we observed that even if the male Large Japanese Field Mouse was in a morphologically agreeable condition amenable to reproductive activity, it was clear that the mouse was not in an endocrinologically reproductive state. In addition, it was revealed that the testicular weight correlated better with plasma testosterone concentration than testicular length. Consequently, we considered testicular weight a better index of reproductive activity than testicular length.

The breeding season of the Large Japanese Field Mouse, therefore, was considered to occur twice in the spring and autumn of each year in this area. TAKANAKA *et al.*[1] reported that the Large Japanese Field Mouse typically fell into roadside gutters from April to June and from August to October. Therefore, we concluded that the season of falling into side gutter was related to the breeding season. The home-range sizes of this mouse during the breeding season were significantly larger than during the non-breeding season[32], this likely results in a heightened breeding season activity relating to the higher side gutter falling incidence for the Large Japanese Field Mouse.

Acknowledgements

We are grateful to the community of Fujinomiya, Shizuoka Prefecture and the staff members of the Fuji farm of the Tokyo University of Agriculture for their support of our field-work.

References

1) TAKANAKA, K., ANDO, M., OGAWA, H., TSUCHIYA, K., YOSHIYUKI, M. and AMANO, T., (2008). Small mammal death in side gutters with constantly flowing water. *Mammalian Science,* **48**, 1–9. (In Japanese)
2) IMAIZUMI, Y., (1962). On the species formation of the *Apodemus speciosus* group, with special reference to the importance of relative values in classification. Part 1. *Bulletin of the National Science Museum,* **5**, 163–259.
3) IMAIZUMI, Y., (1964). On the species formation of the *Apodemus speciosus* group, with special reference to the importance of relative values in classification. Part 2. *Bulletin of the National Science Museum,* **7**, 127–177.
4) TSUCHIYA, K., (1974). Cytological and biochemical studies of *Apodemus speciosus* group in Japan. *Journal of the Mammalogical Society of Japan,* **6**, 67–87. (In Japanese)
5) IMAIZUMI, Y., (1968). Analysis of Ural owl pellet contents. *Zoological Magazine,* **77**, 402–404. (In Japanese)
6) MATSUOKA, S., (1977). Winter food habits of the Ural owl *Strix uralensis* Pallas in the Tomakomai experiment forest of Hokkaido University. *Research Bulletins of the College Experiment Forests Hokkaido University,* **31**, 161–173. (In Japanese)
7) YONEDA, M., ABE, H. and NAKAO, H., (1979). Winter food habits of the Yezo ural owl *Strix uralensis japonica* in a wind shelter-belt. *Journal of the Yamashina Institute for Ornithology,* **11**, 49–53. (In Japanese)
8) MORII, R. and SHIOIRI, T., (1996). On the pellet contents of the Ural owl, *Strix uralensis hondoensis. Kagawa Seibutus,* **23**, 15–20. (In Japanese)
9) SONE, K. and KOHNO, A., (1996). Handling of acorns by Apodemus mice. *Research Bulletin of the Kagoshima University Forests,* **24**, 89–94.
10) ISAJI, H. and SUGITA, H., (1997). Removal of fallen *Aesculus turbinate* seeds by small mammals. *Japanese Journal of Ecology,* **47**, 121–129. (In Japanese)
11) GOTO, S. and HAYASHIDA, M., (2002). Seed dispersal by rodents and seedling establishment of Walnut trees (*Juglans ailanthifolia*) in a riparian forest. *Journal of Japanese Forestry Society,* **84**, 1–8. (In Japanese)
12) SONE, K., HIROI, S., NAGAHAMA, D., OHKUBO, C., NAKANO, E., MURAO, S. and HATA, K., (2002). Hoarding of acorns by granivorous mice and its role in the population of *Pasania edulis* (Makino) Makino. *Ecological Research,* **17**, 553–564.
13) HIRATA, R., TAKAMATSU, N., NAKAMURA, M., FUCHIGAMI, M., HATA, K. and SONE, K., (2007). Secondary dispersal of Pasania edulis acorns by *Apodemus speciosus* into a coniferous plantation. *Journal of Japanese Forestry Society,* **89**, 113–120. (In Japanese)
14) MIYAO, T., MOROZUMI, T. and MOROZUMI, M., (1967). Small mammals of Mt. Yatsugatake, Honshu. VII. Seasonal change in reproduction of the wood mouse, *Apodemus speciosus. Zoological Magazine,* **76**, 161–166. (In Japanese)
15) KONDO, N. and ABE, H., (1978). Reproductive activity of *Apodemus speciosus ainu. Journal of the Faculty of Agriculture, Hokkaido University,* **11**, 160–165. (In Japanese)
16) YOSHIDA, H., (1971). Small mammals of Mt. Kiyomizu, Fukuoka Pref. 3. Reproduction in the Japanese wood mouse, *Apodemus speciosus. Journal of the Mammalogical Society of Japan,* **5**, 123–129. (In Japanese)
17) OH, H.S. and MORI, T., (1998). Histological studies on reproductive organs of the Large Japanese Field Mouse, *Apodemus speciosus. Mammalian Science,* **37**, 103–113. (In Japanese)
18) FUJIWARA, M., (1964). The breeding habit of the Old World Woods Mouse (*Apodemus speciosus speciosus* TEMMINK) in Hiroshima-pref. *Miscellaneous Report of the Hiwa Museum for Natural History,* **7**, 11–14. (In Japanese)
19) USUKI, H., (1966). The seasonal reproductive condition of the old world wood mouse (*Apodemus speciosus*). *Bulletin of the Nagaoka Municipal Science Museum,* **7**, 2–3. (In Japanese)
20) IMAIZUMI, Y., (1970). Reproduction in the Japanese wood mouse in Niigata Pref., Honshu. *Bulletin of the Nagaoka Municipal Science Museum,* **6**, 33–37. (In Japanese)
21) YUKAWA, M., (1971). Reproduction in the Japanese wood mouse (*Apodemus speciosus speciosus* (TEMMINK)) in Hiroshima-Pref. *Miscellaneous Report of the Hiwa Museum for Natural History,* **15**, 1–6. (In Japanese)
22) MURAKAMI, O., (1974). Growth and development of the Japanese wood mouse (*Apodemus speciosus*), I. The breeding season in the field. *Japanese journal of ecology,* **24**, 194–206. (In Japanese)
23) TAKADA, Y., (1983). Life History of Small Mammals in Fallow Fields 5. Reproduction in the Large Japanese Field Mouse and the Feral House Mouse. *Journal of the Mammalogical Society of Japan,* **9**, 246–252. (In Japanese)
24) TATEISHI, T. and SHIRAISHI, S., (1992). Distribution and Reproductive Activity of the Japanese Large Field Mouse, *Apodemus speciosus* around the Lake Sayama, Saitama Prefecture. *Science Bulletin of the Faculty of Agriculture, Kyushu University,* **47**, 85–92. (In Japanese)
25) MAEHATA, I., (1996). Fauna of Muridae, and reproduction and monthly change of the number of *Apodemus speciosus* in the laurel and deciduous forest. *Bulletin of Center for Natural Environmental Education, Nara University of Education,* **1**, 22–32. (In Japanese)
26) JAMESON, E.W., (1950). Determining fecundity in male small mammals. *Journal of mammalogy,* **3**, 433–436.
27) SNEDECOR, G.W. and COCHRAN, W.G., (1989). *Statistical Methods* SNEDECOR G.W. and COCHRAN W.G. eds., The Iowa State University Press. Ames, IA, 217–236.
28) SNEDECOR, G.W. and COCHRAN, W.G., (1989). *Statistical Methods* SNEDECOR G.W. and COCHRAN W.G. eds., The Iowa State University Press. Ames, IA, 83–106.
29) DUNCAN, D.B., (1955). Multiple range and multiple F tests. *Biometrics,* **11**, 1–42.
30) HARTER, H.L., (1960). Critical values for Duncan's new multiple range test. *Biometrics,* **16**, 671–685.
31) DAVID, R., WARREN, B. and KATHLEEN, F., (2001). *Eckert Animal Physiology Mechanisms and Adaptations 5th.,* W. H. Freeman and Co, New York. 344–345.
32) OKA, T., (1992). Home range and mating system of two sympatric field mouse species, *Apodemus speciosus* and *Apodemus argenteus. Ecological Research,* **7**, 163–169.

アカネズミ *Apodemus speciosus* の側溝への落下時期と繁殖期との関連性

高中健一郎*・安藤元一*・天野　卓*・小川　博*

（平成 20 年 11 月 18 日受付/平成 21 年 1 月 23 日受理）

要約：本研究は，アカネズミ *Apodemus speciosus* の側溝への落下時期と繁殖期との関連性を調べることを目的とし，アカネズミの雄の精巣サイズと血漿テストステロン濃度を測定し，繁殖期を調べた。実験に用いたアカネズミの成獣雄は延べ 35 個体であり，静岡県富士宮市において，2003 年 12 月から 2004 年 11 月までに捕獲した。精巣長径の周年変化をみると，2 月から 9 月までの一山型を示し，その期間中の大きさは 12 mm 以上であった。一方，精巣重量および血漿テストステロン濃度の周年変化をみると，共に 4 月と 8 月にピークを持つ二山型を示し，血漿テストステロン濃度の値は 6 月に非繁殖期と同様の低い値まで低下した。アカネズミの雄は，精巣の大きさから性的に活発であると判断された場合でも，内分泌学的には不活発な状態の場合があることが明らかになった。さらに，精巣長径より精巣重量の方がテストステロンとの相関が高いことが認められ，アカネズミの雄の繁殖状態を判断するためには，精巣長径より精巣重量を指標に用いた方が適当であることがわかった。これらのことから，側溝への落下時期は繁殖期の活動と関連していると考えられた。

キーワード：*Apodemus speciosus*, 側溝落下，繁殖期，精巣サイズ，テストステロン

* 東京農業大学大学院農学研究科畜産学専攻