Original paper

Title: **Characteristics of pregnancy following mating at three types of estrus in captive harvest mouse (*Micromys minutus*)**

Reiko Ishiwaka[1,3], Hidetoshi Kakihara[2,3,＊], Yasuhisa Masuda[3]

[1] University of Miyazaki, Faculty of Agriculture, Miyazaki 889-2192, Japan

[2] Tohoku University, Faculty of Agriculture, Sendai 980-0845, Japan

[3] Kuju Grassland Ecomuseum, Fukuoka 810-0033, Japan

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＊To whom correspondence should be addressed. E-mail:

kakihara@kuju-ecomuseum.org

ABSTRACT. Mating in harvest mice, *Micromys minutus*, was observed by introducing males to females that were at cyclic estrus (CE), that had just delivered young (postpartum estrus, PPE), or that were not pregnant but were lactating after PPE (lactational estrus after PPE: LEAP). Copulation leading to weaning of pups occurred at every trial for CE (n = 11) and for PPE (n = 6). In LEAP, estrus with copulation and subsequent pregnancy occurred at each of seven mating trials. At six of these trials, parturition and weaning of pups occurred as well. The mean ± standard error length of gestation was 17.55 ± 0.12 days for mating at CE, 17.56 ± 0.17 days for mating at PPE, and 17.83 ± 0.22 days for mating at LEAP. Generalized linear mixed model analysis found no significant effect of type of estrus at mating on gestation length. Concurrent pregnancy and lactation resulting from mating at PPE or LEAP had no significant effect on the number of young at weaning that were conceived from the mating but also that were on suckling at the time of the mating.

Key words: copulation, gestation length, lactational estrus, mating behavior, parturition

To understand the ecology of a mammal, knowledge of its reproductive traits is essential. The delivery interval is an important life history trait that determines the number of offspring a female can produce in every breeding season and/or her entire lifetime (Gilbert 1984).

With regard to the delivery interval, it is important when and how estrus leading to pregnancy occurs. Estrus in nonpregnant and nonlactating females is known as cyclic estrus (CE). In two rodent families, the Cricetidae and the Muridae, some species, such as the rat *Rattus norvegicus* and the mouse *Mus musculus*, have been shown to have a highly specialized type of estrus (Conaway 1971; Kawata 1983). To maximize the number of offspring produced in a short period of time, this type of estrus has the following characteristics: the length of the estrus cycle is 4 to 5 days, the estrus period is less than 1 day, fertilization only occurs when copulation is close to the time of ovulation, and ovulation is spontaneous. As in animals with this type of estrus, the harvest mouse is a highly prolific, small-sized prey species with a short life span. It is polyestrous (Trout and Harris 2008), with CE at an interval of 4 to 5 days (Harris 1979). Little information is available on the context of copulation during mating.

Females of most rodent species have another type of estrus, postpartum estrus (PPE), in which females become receptive and can mate shortly after giving birth (Bateman 1957; Dewsbury 1967; Gilbert 1984). Mating at PPE has the advantage of reducing the delivery interval. Harris (1979) and Trout and Harris (2008) reported copulation on the day after parturition and ensuing pregnancy in the harvest mouse.

Ovarian function is suppressed during lactation in humans, domestic animals, laboratory animals, and wild animals (McNeilly 2001). As a result, if a female misses the opportunity to mate and become pregnant at PPE, the lactation period will extend the delivery interval. Another type of estrus in rodents has been reported by Gilbert (1984). This type occurs in some species during the lactation period after PPE ceases (which we abbreviate as LEAP). This type of estrus exists only in species of the family Cricetidae, particularly in members of the subgenus Arvicolinae (originally Microtinae). For the harvest mouse, there are no experimental or field data that indicate the possibility of mating and pregnancy except at PPE during the lactation period of 15 to 16 days (Trout and Harris 2008). We conducted this study to confirm the possibility of pregnancy at LEAP in the harvest mouse, since Gilbert (1984) suggested that LEAP contributes to maximizing the number of possible offspring in some species with a short life span and very small size.

Gestation length is another important element of the delivery interval. The gestation period in mammals has a strong relationship with body size and is shorter in smaller animals (e.g., Kihlström 1972; Martin and MacLarnon 1985). The duration of gestation in the harvest mouse, which weighs about 6 g as an adult (Trout and Harris 2008), is reported as 17 to 19 days, which is very short compared with other murid species (Ishiwaka and Mōri 1999; Trout and Harris 2008). These data were obtained for pregnancies following mating at CE. As observed in the rat and the mouse, the extension of gestation length following mating at PPE was indicated by Harris (1979) in the harvest mouse. The prolonged period of gestation avoid the heavy burden of concurrent lactation and pregnancy in female parents (Norris and Adams 1981; Johnson et al. 2001; Rutkowska et al. 2011). Therefore, the length of gestation following mating at LEAP, if it occurs, should be compared with the length of gestation following mating at CE and at PPE.

In this study, we examined whether copulation and pregnancy in the female harvest mouse while caring for her pups can occur during the remaining lactation period after the end of PPE (LEAP). We then compared the lengths of the gestation periods following mating at CE, at PPE, and at LEAP. To calculate the length of gestation, the time of copulation should be confirmed. Since there is little information on the mating behavior of the harvest mouse, we analyzed video recordings to observe the mating process from the introduction of the male and tried to identify copulation accompanied by ejaculation. We also attempted to determine the delivery time more accurately (not only by date) based on the time that the female went into and out of her breeding nest. It was necessary to know the effects of concurrent sexual and maternal behavior and concurrent lactation and pregnancy (mating at PPE and at LEAP) on the growth of fetuses and suckling pups. We examined the number of young at weaning that were conceived from mating at CE, PPE, and LEAP and that were on suckling at the time of mating at PPE and LEAP.

Methods

Animals

We used 11 females and five males from a colony that was established in 1990 based on six founder individuals caught in Saga, Kyushu, Japan, and maintained for about 25 generations. As females of this species are reported to have given birth at 62 days of age (Trout and Harris 2008), the individuals used ranged from 60 to 210 days old.

The mice were usually kept individually in plastic cages (20 cm width, 23 cm length, and 16 cm depth) that were supplied with wood chips on the floor, rice straw for nesting material, a feeder, and a water bottle. The cages were kept in a room with natural light from windows under uncontrolled room temperature in Fukuoka, Kyushu, Japan (130°23′E, 33°34′N). The mice were given water and food, consisting of rice mixed with hempseed in a one-to-one ratio, along with commercial mouse food (MF type, Oriental Yeast Co.), *ad libitum.*

Observations of mating behavior

The structure of the observation cage was as follows. Three sides of the cage were made of clear acrylic boards, and the other side was made of plywood. The ceiling and floor of the cage were made of wire netting (5 mm square mesh). The cage was 50 cm in width, 40 cm in length, and 40 cm in height. We set up a bunch of green shoots of grasses (*Imperata cylindrica* var. *koenigii* in the summer and autumn and *Lolium multiflorum* and *Avena sativa* in the winter and spring) about 40 cm in length vertically into the wire netting in a fourth part of the floor, so that the pregnant mouse could make a spherical nest 25 to 30 cm above the floor on the standing shoot. The rest of the floor was covered with straw, as in the animals' natural condition. The light and temperature conditions during observation were the same as those during usual breeding. Mating trials were conducted, except for the high-temperature season in summer. The mice were thus observed under temperatures ranging from 5 °C to 30 °C. Mice that were under observation were given the same food as described above with water *ad libitum* using a feeder and a bottle. The handling and care of the animals were conducted according to the *Guide for the Care and Use of Laboratory Animals* (Institute for Laboratory Animal Research 2010).

For regular CE mating, an adult, nonpregnant, nonlactating female was placed in the observation cage either 1 day or half of the day before an adult male was introduced. Eleven pairing trials were conducted using 10 females and five males. For PPE mating, we placed a pregnant female in the observational cage and gave her enough time to build a breeding nest and to prepare for parturition and rearing pups. A male was introduced around the day of parturition, and we defined estrus as PPE for copulation that occurred within 24 h after parturition. The data were obtained by six pairings using four females and three males. For the third kind of mating, we introduced a male one time between 1.27 and 8.06 days after parturition, when PPE had ceased. These matings were conducted to examine the possibility of the occurrence of estrus

(LEAP) and pregnancy in a lactating female. Seven pairings were conducted using five females and three males. The male was removed from the cage within 6 h after copulation was observed.

We defined the behavior of a male grooming his own abdominal region (genital grooming) after mounting a female as indicating an occurrence of copulation with ejaculation. Because we could not determine when parturition occurred during the female's stay in the nest, we defined the time of parturition as the midpoint of the period between the time the female last entered her nest before parturition and the time she first left the nest after parturition.

To avoid disturbing the females, we video-recorded the behavior of the mice using SD card recorders, AD-S20 type (Carrot Systems Co.) and DVF-7 type (Corona-Dengyo Co.), and high-definition miniature cameras with infrared irradiation devices (an MK-323 type f = 2.5 mm wide-angle lens and an ITC-

405HIR type f = 8 mm lens). The movements of the mice were recorded using a motion recording system (30 frames per second).

The camera was set to record behavior from the time of introduction of the male until his removal after copulation. For the determination of parturition time, the camera was operated from 2 days before the supposed parturition day (18th day from copulation) until pups left the nest. Because objects in the cage blocked part of the movie shot, we were unable to record all the mating behavior performed in the cage and to determine which copulation led to pregnancy. Therefore, we used the time when copulation was first confirmed by the video during each mating session for calculation of the gestation period and of the time from the introduction of the male to copulation.

Statistical analysis

To elucidate the effects of mating at CE, PPE, or LEAP on the length of gestation and the time from the introduction of a male to the occurrence of copulation, a generalized linear mixed model (GLMM), assumed to have a Gaussian errors structure with an identity link function, was applied. In these GLMMs, we included as explanatory variables the type of estrus at which pregnancy occurred (CE, PPE, or LEAP) and the season when the copulation took place (the long- or short-day season according to the spring and autumn equinox) as fixed effects. Because individual mice were repeatedly observed, we also included individual female as a random factor. To examine the effects of a mother's pregnancy on her suckling pups and a mother's lactation on her fetus, GLMM analysis assuming a Poisson errors structure was conducted using the above explanatory factors. For the response factor in the former GLMM, the number of young at weaning produced by mating at CE and the number of suckling pups when a mother became pregnant at PPE or LEAP were used. With regard to the latter GLMM, we used the number of young at weaning produced by mating at CE and the number of young produced by mating at PPE or LEAP as response factors. All analyses were conducted using R: Language and Environment for Statistical Computing Version 2.4.0 (R Development Core Team 2017) with the lme4 package (Bates et al. 2015), and statistical significance was determined by the Wald chi-square test. The data are expressed as means ± standard error.

Results

In mating trials in the later part of the lactation period when PPE had ceased (LEAP), all seven trials resulted in copulation and pregnancy, and pups were successfully weaned in six of them (Table 1). In these trials, after the male was introduced, the behavior of the lactating female shifted gradually to her behavior at estrus, and copulation then occurred in 0.02 to 2.09 days (mean, 0.61 ± 0.32 days) after the introduction of the male (3.36 to 8.83 days after parturition). The mean duration of gestation was calculated as 17.83 ± 0.22 days according to the results from six trials with weaning accomplished.

In the mating trials at CE of a nonpregnant and nonlactating female, copulation and pregnancy occurred in all 11 trials (Table 2). Copulation was observed 0.01 to 3.02 days (mean, 0.91 ± 0.32 days) after the introduction of the male. The mean period between copulation and parturition, i.e., the duration of gestation, was 17.55 ± 0.12 days.

Table 3 shows the results of the trials in which the male was introduced to the female around the time of parturition, i.e., around PPE. Copulation and pregnancy occurred in all six trials. Copulation occurred 0.08 and 0.13 days after parturition in two trials in which the male was introduced before parturition. In the other four trials, in which the male was introduced after parturition, copulation occurred 0.03 to 0.60 days after the introduction of the male (0.12 to

0.80 days after parturition). The mean duration of gestation was 17.56 ± 0.17 days.

The results of the GLMM tests are presented in Tables 4 and 5. There were no significant effects of the type of estrus (CE, PPE, or LEAP) at which copulation occurred on gestation length (χ^2 = 1.537, P = 0.215), the time from the introduction of the male until copulation (χ^2 = 0.622, P = 0.440), or the number of young at weaning (χ^2 = 0.549, P = 0.459). When we examined the effect of the mother's pregnancy on her suckling pups (pregnancy at PPE or LEAP), no significant effect was observed on the number of young at weaning (χ^2 = 0.008, P = 0.927). There was no significant effect of day length on gestation length (χ^2 = 1.082, P = 0.313), the time from the introduction of the male until copulation (χ^2 = 0.709, P = 0.410), the number of young at weaning that were conceived from the mating (χ^2 = 0.781, P = 0.377), or the number of young at weaning that were on suckling when the mating trial was conducted ($\chi2$ = 0.001, P = 0.987).

Discussion

Behavior analysis by video recordings

On the basis of the preliminary information on the behavioral interactions associated with mating obtained by video recordings, we defined the following series of behaviors as an occurrence of copulation with ejaculation: first, the male mounted the female while she assumed the lordosis posture; and second, the male groomed his own abdominal region (genital grooming) after leaving the female, as described in the rat (Hull and Dominguez 2007). The very high probability of pregnancy in all the mating trials indicates that this definition is correct. Copulation was observed once in each mating trial at PPE and several times at intervals of 2 to 3 h in the mating trials at the other two types of estrus. Because we set up a bunch of green shoots of grasses in the cage to provide a place for nesting, it was impossible to observe and record all the actions performed inside it and to determine which of several copulations led to pregnancy. Therefore, we used the time when copulation was first confirmed by the video during each trial for calculation of the length of the gestation period and of the time from the introduction of the male until copulation. On the assumption that ejaculation might have occurred during each copulation, copulation followed by pregnancy could have occurred up to 3 h after the estimated time.

According to our observation, a female in late pregnancy leaves her nest every 1 to 2 h and visits the feeder to eat. As the time of delivery approaches, she stays longer in the nest. The length of stay in the nest is influenced by whether it occurs during the day or at night, the season, litter size, and the number of times the female has given birth. Therefore, we also confirmed whether parturition had occurred by the change in the shape of the female's abdomen in video recordings. We could not determine at which point during the female's stay in the nest parturition occurred, and we therefore assumed that the midpoint of the stay was the time of parturition. In the videos, most of the stays for parturition lasted 3 to 5 h. Therefore, parturition took place during the range of 2.5 h before and after the estimated time. By integrating both error ranges of mating time and delivery time, the estimated duration of gestation has an error of +2.5 h (0.1 days) or −5.5 h (0.23 days).*Mating at CE*

Copulation was observed from 0.01 to 3.02 days after the introduction of the male (Table 2). This suggests the possibility that the female harvest mouse has the same type of regular estrus cycle as the laboratory rat and mouse, in which CE occurs every 4 to 5 days (e.g., Chaffin and VandeVoort 2013). However, there has not been much of ethological research on CE in the harvest mouse to prove this, such as was conducted by Brandt (2010). We could not determine at which stage of an estrus cycle an observed female was at the start of our experiments. Therefore, some females might not have been in an estrus stage, or their estrus cycles might have been reset upon contact with a male (male-induced estrus), such as occurs in mice, rats, and voles (e.g., Petrulis 2013). Further research is needed to clarify the length of the estrus cycle and the estrus-inducing effect of contact with a male in the harvest mouse.

Mating at PPE

Pregnancy and parturition occurred in all the trials of mating at PPE (Table 3). It is well known that in species such as the laboratory mouse (Bronson et al. 1966) and the prairie vole, *Microtus ochrogaster* (Witt et al. 1990), the probability of pregnancy from mating at PPE is high. In this study, the females attacked approaching males when the males were introduced 24 h or more after parturition, indicating the end of PPE. Therefore, we defined copulation occurring within 24 h after parturition as mating at PPE. Ovulation at PPE occurs 12 to 18 h after parturition in the laboratory mouse (Berry 1970) and 3 h after parturition in the Norway rat, *Rattus norvegicus* (Connor and Davis 1980). We have no way to estimate the time of ovulation, but it is certain that PPE begins very soon after parturition and terminates soon after copulation, which occurs within a short period. In the trials in which the male was waiting for the female to come out of the nest to feed after parturition (two of six trials), copulation occurred the first time she left the nest. Immediately after copulating, the female returned to the newborn pups in the nest. When the female left the nest for the second time about an hour later, she behaved aggressively toward the male. In other trials in which the male was introduced 0.02 to 0.2 days after the delivery, the female accepted copulation within a short interval (Table 3) and then immediately returned to the nest to care for her pups.

Mating in a later part of the lactation period after PPE ceases

The approach of a male in the later part of the lactating period, after PPE had ceased, led the female to estrus (LEAP), copulation, and pregnancy. Mating at LEAP as well as at PPE resulted in pregnancy concurrent with lactation, with a high probability of successful reproduction (Table 1). If a female rat or mouse does not become pregnant during PPE, the next estrus and pregnancy can occur only after lactation ceases and the estrus cycle is resumed (Mantalenakis and Ketchel 1966). During the lactation period of a nonpregnant female house mouse, the level of estrogen decreases gradually after PPE, and then the level of progesterone increases slowly. This is probably the cause of suspension of the estrus cycle during lactation (Hansen et al. 1983). Suppression of folliculogenesis by secretion of prolactin stimulated by suckling is also related to the inhibition of continuous reproduction (Taya and Greenwald 1982; Bachelot and Binart 2007).

The length of the period when the female was aggressive toward the male after PPE varied with the time after parturition. Despite large variation in the length of time from the introduction of the male until his acceptance by the female, copulation was observed in every mating trial. GLMM found no significant effect of estrus type (CE, PPE, or LEAP) on the time from male introduction to copulation (Table 5). The larger variations in copulation time for mating at CE and LEAP might have caused insignificant statistical effects. Although the results of this experiment presume resumption of the estrus cycle after parturition in this species, detailed endocrinological studies are needed for additional clarification.

When mating occurred at LEAP or PPE, the suckling pups in the nest were at the age of 3.36 to 8.83 days or less than 1 day, respectively. In one of seven trials of mating at LEAP, no pup was weaned, although copulation and parturition were confirmed. Further investigation is needed to determine whether the sexual behavior of a female during the lactation period may lead to her failure to care for the pups.

Length of gestation

The length of gestation following mating at CE in the harvest mouse has been reported as 17 to 19 days (Trout and Harris 2008) and 19 days (Ishiwaka and Mōri 1999). Because our calculations contain an error related to the estimates of copulation time and parturition time, as described above, the length of gestation in this study is not considered to be markedly different from those already reported.

An extended length of gestation at PPE has been reported in many rodents, such as the rat (Woodside et al. 1987), the laboratory mouse (Norris and Adams 1981), and the Campbell hamster, *Phodopus campbelli* (Roy and Wynne-Edwards 1995). The prolonged period of gestation due to delayed implantation is considered to occur to avoid excessive demands on the mother from the fetuses and suckling pups (Norris and Adams 1981; Johnson et al. 2001; Rutkowska et al. 2011). Harris (1979) stated that in the harvest mouse, the length of gestation following copulation at PPE was extended to 3 weeks. We did not, however, obtain any data supporting this report. No delay was found in any pregnancies after mating at PPE (Table 3) or LEAP (Table 1). Our females were allowed *ad libitum* feeding so that they could ingest enough food for their suckling pups and fetuses. Further investigation under field conditions will be necessary to understand the harvest mouse's strategy to overcome the load of concurrent pregnancy and lactation.

Information on factors influencing the length of gestation, such as day length in mice (Lanman and Seidman 1977), is not available for the harvest mouse. GLMM in our study did not show any significant effects of day length on the length of gestation or on the time from the introduction of the male to copulation (Table 4)

Number of young at weaning

Litter sizes of one to seven (mean, 4.8) were reported for the harvest mouse (Trout 1978) and of 5.42 ± 0.23 throughout 1 year in Kyushu, Japan (Shiraishi 1962). The number of young at weaning in our study ranged from one to seven. Concerning concurrent pregnancy with lactation, there are two conflicting reports: a reduction in litter size that was conceived by the mating (Oswald and McClure 1990), and no difference exists in litter size between the one that was conceived from the mating and that was lactating at the mating (Gilbert et al. 1983). This discrepancy may be related to the amount of food resources, which must simultaneously meet the demands of both the suckling pups and the fetuses. GLMM found no significant effects of the type of estrus or of day length on the number of young at weaning that were conceived from the mating and that were on suckling at the time of mating (Table 5). However, since we used the number of young at weaning to avoid affecting the behavior of lactating females by counting pups in her nest, it is undeniable that a difference in litter size at birth could exist between pups conceived at different types of estrus. Further research on body weight and the ratio of male to female pups is necessary.

Conclusions

The lactation period of the harvest mouse is 15 to 16 days, which is shorter than that of other small rodents (Ishiwaka and Mōri 1999). If the duration of gestation is assumed to be 18 days, the delivery interval would be 33 to 34 days, if copulation occurs directly after the end of the lactation period. Postpartum pregnancies permit far higher delivery frequency (every 17 to 18 days) over the course of a female's lifetime. Small mammals should have evolved to deliver as many offspring as possible in a lifetime. Success in pregnancy at PPE or LEAP could result in a high level of fitness. In this study, one female gave birth to five litters within 75 days from October 6 to December 20.

Although mating at LEAP and its succeeding pregnancies have been proved in this study, there is no information on how frequently pregnancy after mating at PPE or LEAP occurs in the field or on how pregnant and lactating females encounter males in the field. Further study is needed to clarify the significance of the possibility of pregnancy from mating at PPE or LEAP in the life history of the harvest mouse.

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TABLE TITLES

Table 1. Gestation length, time from the introduction of the male until copulation, and number of young at weaning in mating of harvest mouse females at estrus occurring in the later part of the lactation period after postpartum estrus (LEAP). Data were obtained from seven pairings using five females and three males.

Table 2. Gestation length, time from the introduction of the male until copulation, and number of young at weaning in mating of harvest mouse females at a regular cyclic estrus (CE). Data were obtained from 11 pairings using 10 nonpregnant, nonlactating females and five males.

Table 3. Gestation length, time from the introduction of the male until copulation, and number of young at weaning in mating of harvest mouse

females at postpartum estrus (PPE). Data were obtained from six pairings using four females and three males.

Table 4. Estimated parameters of the generalized linear mixed model (with Gaussian errors) for length of gestation and time from the introduction of a male until copulation. The fixed factors are the three mating times—CE, PPE and LEAP—and the mating season (long-day or short-day season). Individual female is a random factor.

Table 5. Estimated parameters of the generalized linear mixed model (with Poisson errors and a logit-link function) for the number of young at weaning that were conceived from mating at CE, PPE, and LEAP and that were on suckling at mating at PPE and LEAP. The fixed factors are the three mating times—CE,

PPE and LEAP—and the mating season (long-day or short-day season).

Individual female is a random factor.

Table 1.

Female individuals[1]	Time from previous parturition until introduction of a male (days)	Number of suckling pups counted at weaning[2]	Date and time of copulation[3]	Time from introduction of the male until copulation (days)	Time from previous parturition until copulation (days)	Date and time of parturition[4]	Number of young at weaning	Gestation length (days)
A	4.90	3	2015/1/3 9:39	0.13	5.03	2015/1/22 1:30	5	18.66
K	8.06	2	2015/2/6 7:51	0.76	8.83	2015/2/23 10:23	4	17.11
K	7.98	4	2015/3/3 23:25	0.56	8.54	2015/3/21 21:17	1	17.91
C	1.27	3	2015/10/9 12:13	2.09	3.36	2015/10/27 6:30	3	17.76
F	5.41	6	2016/4/5 7:02	0.03	5.44	2016/4/22 17:50	6	17.45
F*	5.73	6	2016/4/29 18:06	0.02	5.84	2016/5/17 0:00	0	18.09
H	6.99	3	2016/4/30 8:15	0.11	7.01	2016/5/18 10:25	4	18.09
Average(±*SE*)[5]	5.56(1.02)	3.5(0.6)		0.61(0.32)	6.17(0.87)		3.8(0.7)	17.83(0.22)

[1]A, K, C, F and H represent female individuals. Individual of the same alphabet in Tables represent the same individual.

[2]Number of suckling pups of the female at mating counted at weaning.

[3]Date and time of the first copulation.

[4]Time of the midpoint of the stay in a breeding nest for parturition.

[5]Calculated excluding the data of F*.

Table 2.

Female individuals[1]	Date and time of copulation[2]	Time from introduction of the male until copulation (days)	Date and time of parturition[3]	Number of young at weaning	Gestation length (days)
A	2/16/2015 1:08	0.17	2015/3/5 5:20	3	17.17
B	6/2/2015 12:20	2.14	2015/6/20 21:20	3	18.38
C	9/19/2015 0:11	1.26	2015/10/6 3:36	3	17.14
D	11/3/2015 8:40	0.61	2015/11/21 9:30	2	18.03
E	12/14/2015 10:00	3.02	2016/1/1 4:35	3	17.77
F	1/7/2016 22:21	2.18	2016/1/25 5:19	2	17.29
G	2/15/2016 12:15	0.09	2016/3/4 7:19	4	17.79
F	3/13/2016 14:20	0.14	2016/3/30 20:25	6	17.25
H	4/6/2016 20:55	0.39	2016/4/24 12:00	3	17.63
I	5/17/2016 22:22	0.05	2016/6/4 9:00	4	17.44
J	12/22/2016 2:11	0.01	2017/1/8 5:22	3	17.13
Average (±*SE*)		0.91（0.32）		3.27(0.33)	17.55(0.12)

[1]A–J represent female individuals. Individual of the same alphabet in Tables represent the same individual.

[2]Date and time of copulation first confirmed in video recording.

[3]Time of the midpoint of the stay in a breeding nest for parturition.

Table 3.

Female individuals[1]	Date and time of previous parturition	Number of suckling pups at weaning[2]	Date and time of copulation[3]	Time from previous parturition until copulation	Time from introduction of the male until copulation	Date and time of parturition[4]	Number of young at weaning
				(days)	(days)		
A	2015/3/5 5:20	3	3/6/2015 0:30	0.80	0.60	2015/3/23 9:45	5
K	2015/6/18 9:30	3	6/18/2015 21:10	0.49	0.47	2015/7/5 23:50	4
C	2015/10/27 6:30	3	10/27/2015 13:52	0.31	0.29	2015/11/14 14:20	1
C[5]	2015/11/14 14:20	1	11/14/2015 17:22	0.13	0.91	2015/12/2 19:20	3
C[5]	2015/12/2 19:20	3	12/2/2015 21:12	0.08	0.27	2015/12/20 0:30	7
F	2016/1/25 5:19	2	1/25/2016 8:10	0.12	0.03	2016/2/11 23:30	5
Average(±*SE*)		2.5(1.0)			0.27(0.09)[6]		4.2(0.8)

[1]A, K, C and F represent female individuals. Individual of the same alphabet in Tables represent the same individual.

[2]Number of suckling pups of the female at mating counted at weaning.

[3]Date and time of copulation first confirmed in video recording.

[4]Time of the midpoint of the stay in a breeding nest for parturition.

[5]Males introduced before the delivery of pups.

[6]Time from the previous parturition until copulation is used in calculation for C[5].

Gestation length
(days)
17.39
17.11
18.02
18.08
17.14
17.64
17.56(0.17)

Table 4.

Response factor	Model	Estimate (Standard error)			Deviance	AIC	Chi-square	Chi-square *df*	*P*(>Chi-square)
		β_0	β_1	β_2					
Gestation length	$\beta_0 + r$	17.63 (0.09)			27.01	33.01			
	$\beta_0 + \beta_1 E + r$	17.78 (0.16)	−0.13 (0.11)		25.52	33.52	1.490	1	0.222
	$\beta_0 + \beta_1 E + \beta_2 S + r$	17.85 (0.17)	−0.14 (0.11)	−0.21 (0.21)	24.39	34.39	1.130	1	0.288
Time from introduction of the male until copulation	$\beta_0 + r$	0.67 (0.18)			57.49	63.49			
	$\beta_0 + \beta_1 E + r$	0.43 (0.32)	0.19 (0.22)		56.66	64.66	0.825	1	0.364
	$\beta_0 + \beta_1 E + \beta_2 S + r$	0.54 (0.34)	0.18 (0.22)	−0.37 (0.41)	55.77	65.77	0.895	1	0.344

E, the type of estrus mated (CE, PPE, LEAP); S, the mating season (long-day season, short-day season);
β_0, intercept; β_1 and β_2, the parameters of the fixed effects; r, the random effect for individual females.

Table 5.

Response factor	Model	Estimate (Standard error)			Deviance	AIC	Chi-square	Chi-square *df*	*P*(>Chi-square)
		β_0	β_1	β_2					
The number of young at weaning that were concieved by the mating*	$\beta_0 + r$	1.14 (0.12)			77.19	81.19			
	$\beta_0 + \beta_1 E + r$	1.13 (0.16)	0.01 (0.14)		77.19	83.18	0.009	1	0.926
	$\beta_0 + \beta_1 E + \beta_2 S + r$	1.30 (0.18)	0.01 (0.14)	0.00 (0.26)	77.18	85.18	0.001	1	0.987
The number of young at weaning that were on suckling at the mating**	$\beta_0 + r$	1.30 (0.11)			85.70	89.70			
	$\beta_0 + \beta_1 E + r$	1.22 (0.15)	0.09 (0.13)		85.23	91.23	0.472	1	0.492
	$\beta_0 + \beta_1 E + \beta_2 S + r$	1.28 (0.17)	0.10 (0.13)	−0.22 (0.25)	84.42	92.42	0.808	1	0.369

E, the type of estrus mated (CE, PPE, LEAP); S, the mating season (long-day season, short-day season);
β_0, intercept; β_1 and β_2, the parameters of the fixed effects; r, the random effect for individual females.

*the response variable is the number of young at weaning that were conceived from mating,

**the response variable is the number of young at weaning that were conceived at mating at CE, and the number of young at weaning that were on suckling at mating at PPE or LEAP.