Influence of increasing number of mating partners on mating behaviour and reproduction in Brandt's voles

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A b s t r a c t . The influence of increasing number of mating partners on the copulatory behaviour and reproduction in Brandt's voles (*Microtus brandti*) was studied. Compared with the control group of 1 ♂ + 1 ♀, our results showed when increasing only female partners, the mating opportunity was increased in males while decreased in female in the treatment group of 1 ♂ + 2 ♀♀; the mating opportunity of male and female were both decreased significantly in the treatment group of 1 ♂ + 3 ♀♀. When increasing only male partners, male mating opportunity in treatment groups 2 ♂♂ + 1 ♀ and 3 ♂♂+ 1 ♀ decreased significantly while no change was significant in females in both treatment groups. When increasing the number of male and female partners simultaneously in the treatment group of 2 ♂♂ + 2 ♀♀, the mating opportunity for both males and females was decreased significantly. Comparing with the control, the mean litter size in all five-treatment groups lessened significantly and female pregnancy rate dropped significantly if they copulated less than five times. From these results, we have demonstrated a significant reproductive interference due to mate competition in Brandt's voles and thus, competitively reproductive interference may be an important behavioural mechanism in regulating population density in this rodent species. However, due to possible laboratory artifacts, we still need further study, especially in the field, to validate our results.

Key words: density, operational sex ratio, Brandt's vole (*Microtus brandti*)

Introduction

The intensity of sexual selection varies with socio-ecological conditions (A r n o l d & W a d e 1984). Changes in the number of mating partners have a profound effect on animals' demography and behaviour via changes in the operation sex ratio (OSR) and the density of breeding animals. This, in turn, will influence mate competition, reproductive tactics and the mating system (E m l e n & O r i n g 1977, F u e n t e s & D e w s b u r y 1984, O w e n s et al. 1994, N e l s o n 1995, R e y n o l d s 1996). Studies on sexual selection have demonstrated that mate competition will typically intensify when the OSR deviates from 1 : 1, especially when the OSR is skewed to males in rodents (e.g., K v a r n e m o et al. 1995, P a r k e r & S i m m o n s 1996, M i c h n e r & M c l e a n 1996).

Field observations have revealed that the OSR (male to female) in the cape ground squirrel (*Xerus inauris*) is 10 : 1, and males compete for females through repeated

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copulation and mating inference (W a t e r m a n 1998). In the field vole (*Microtus agrestis*) in enclosures, when the female density is stabilized, increasing male number leads to the shrinkage and greater overlap of home ranges and thus presumably intensifies the competition among males (N e l s o n 1995). In the masked palm civet (*Paguma larvata*), males, when the number is artificially increased, begin to interfere with one another when one of them is mating with a female (J i a 2000). Mating interference is also found in the Tibetan macaque (*Macaca thibetana*) and the frequency of mating inference is higher in adults than in young (X i o n g 1993) for both in male and female.

The Brandt's vole (*Microtus brandti*) inhabits the vast steppe habitat, stretching from the Baikal area in Russia, Mongolia to Inner Mongolia in China (S h i 1988, L i u et al. 1994). It usually breeds from late March to August. Its population density fluctuates greatly between years. Outbreaks of voles occur irregularly, every 5–7 years. The mating system of Brandt's vole is still in debate, but most studies infer monogamy and polygamy (Z h a n g & Z h o n g 1981, F a n g & S u n 1991, X i e et al. 1994, W a n et al. 1998, Z h a n g & Z h a n g 2004). Brandt's vole is considered a major pest because it may destroy grassland, compete for food with livestock and spread diseases (W a n et al. 1998). Thus reducing the rodent population to a desirable level is a challenge to population biologists and wildlife managers. The traditional control method using rodent poisons incurs several significant side effects (Z h o n g et al. 1999). Hence, seeking environmentally friendly, alternative methods to control the rodent pest becomes increasingly important. Fertility control is among such attempts. Theoretical modeling (S h i et al. 2002) has shown that it may be a better method than using poisons to control Brandt's vole populations. One of the key issues is the behaviour of the animal because mating interference and mating system influence the efficacy of fertility control in managing populations (C a u g h l e y et al. 1992, Z h a n g 2000). For this, Z h a n g (2000) proposes the notion of *Competitively Reproductive Interference*, which refers to how well sterilized individuals may compete with normal individuals for resources, especially mates, thus reducing the normal individuals' mating opportunity, so curbing the population. Useful as the idea may appear, no study, has so far been attempted to confirm it.

This study, by manipulating the number of mating partners and observing consequent behaviour change, is aimed at measuring the above mentioned competitively reproductive interference in Brandt's vole. We hypothesize that the mating opportunity of Brandt's vole declines as the number of mating partners increases as a consequence of intensified mate competition. The results will be clearly valuable to the application of the fertility control method for harnessing Brandt's vole population growth.

Material and Methods

We tested our hypothesis by using the following experimental design. To manipulate the OSR and density in our experiment, we established six treatment groups in our study. Control group (Group I) had one male paired with one female (1 ♂: 1 ♀). Experimental groups included Group II with one male paired with two females (1 ♂: 2 ♀♀) and Group III with one male paired with three females (1 ♂: 3 ♀♀), Group IV with two males paired with one female (2 ♂♂: 1 ♀), Group V with three males paired with one female (3 ♂♂: 1 ♀), and Group VI with two males paired with two females (2 ♂♂: 2 ♀♀). There were ten replicates for each treatment. Each animal was used only once.

All experimental voles were from the offspring of a wild population caught in July and August in 2000. They were reared separately in plastic boxes (28.5 × 17.2 × 15.8 cm) in the laboratory and were provided with food and water *ad libitum*. The photoperiod was set at 16L: 8D, and the light period began at 05:00. Dim red light shone at all times. The temperature was set at 25 ± 1℃. Only mature and healthy individuals were selected for the experiment based on the degree of testis descendance or the open condition of the vagina. This ensured that both males and females were mature and ready to mate and that, for females, they were in estrus and receptive. Males and females were premated, and only those that showed mounting and receptivity were selected for use. The individuals selected to mate were from different lineages. The mean body size was (41.6 ± 5.1 SD) g of male ($n = 100$) and (38.3 ± 6.2 SD) g of female ($n = 100$), respectively, and males and females did not differ significantly in body size ($t = 1.640$, $df = 198$, $P = 0.115$).

We observed the mating behaviour of the vole in an observational box. The observational box was made by juxtaposing two plastic boxes (each having the size of 37 × 27 × 17 cm) together, with a hole (diameter 15 cm) in the joined sides. A piece of transparent glass was used to cover the box to prevent the escape of the animals. We held male and female voles separately before observation. By using agonistic behaviour between males, we identified the winner as the dominant male while the loser was the subordinate male in dyadic interactions for group IV and group VI. We recorded behavioural patterns and collected data through a kinescope monitor. The sampling was recorded continuously, and the entire observation period was set at one hour following D e w s b u r y (1972). We identified individuals by cutting out unique patterns on the fur. After the completion of each observation period, the box was first rinsed using tap water, then wiped clean with alcohol to remove residual odors.

A mating series in the vole includes mounting, penis insertion, pelvic thrusting and ejaculation. We collected the following data about mating behaviour: (a) mating frequency (MF), referring to the number of copulations that occurred within one hour, (b) mating duration (MD), referring to the length of each copulation in seconds, (c) mating interval (MI), referring to the time between two consecutive copulations in seconds, and (d) thrust frequency (TF), referring to the number of pelvic thrust per second during a copulation in male. For all data, we did not distinguish whether the vole copulated with the same or different mates.

We tested the normality of data distribution by using a one–sample Kolmogorov-Smirnov test. Independent samples t-test (when data distribution was normal) or Mann-Whitney test (when the normality prerequisite was violated) was used for testing the significance in the difference between two treatment groups. We used Wilcoxon paired-sample signed ranks test when comparing with paired data from two groups. One-Way ANOVA (when neither the normality nor equal variances prerequisite was violated) or Kruskal-Wallis test (when either the normality or equal variances prerequisite was violated) was used for testing significance in the difference for three treatment groups. LSD (for parametric ANOVA) or Games-Howell (for non-parametric ANOVA) was used for post hoc multiple comparisons after ANOVA. We used Spearman rank correlation coefficient for analyzing the association between some behaviours measured. Chi-square test was used to test the distribution of pregnancy rate related mating frequency. The significance level for all tests was set at $\alpha = 0.05$.

Results

Influence of the number of mating partners on copulation

Increasing the number of female partners only. It included group I (1 ♂ + 1 ♀), group II (1 ♂ + 2 ♀♀) and group III (1 ♂ + 3 ♀♀) in our analysis. For males (see in Fig. 1 a), there was a significant difference in mating frequency ($F_{2, 27} = 7.36$, $P = 0.002$) and thrusting frequency ($F_{2,24} = 32.6$, $P = 0.00$). No significant difference existed in mating duration ($F_{2, 24} = 1.80$, $P = 0.19$) and mating interval ($F_{2, 24} = 1.2$, $P = 0.31$). Multiple comparisons (Games-Howell) showed no significant difference in mating frequency in males between group I and group II ($P = 0.362$), while male mating frequency decreased significantly in group III compared with groups I and II ($P = 0.045$; $P = 0.004$). Thrusting frequency was highest in group II, then in group III, and was lowest in group I (between groups I and II: $P = 0.00$; between groups I and III: $P = 0.00$; between groups II and III: $P = 0.02$. LSD was used). For female voles (see in Fig. 1 b), the difference in mating frequency was significant (Kruskal-Wallis test, $X^2 = 27.2$, $df = 2$, $P = 0.00$). No difference was found between group I and group II ($t = 1.1$, $df = 28$, $P = 0.28$), but group III was significantly lower compared with groups I and II (Mann-Whitney test, $U = 14$, $P = 0.00$; $U = 104.0$, $P = 0.00$, respectively). One-Way ANOVA test showed no difference in mating duration and mating interval ($F_{2, 36} = 0.99$, $P = 0.38$; $F_{2, 32} = 0.39$, $P = 0.68$),

Increasing the number of male partners only. It included group I (1 ♂ + 1 ♀), group IV (2 ♂♂ + 1 ♀) and group V (3 ♂♂ + 1 ♀) in our analysis. For males (see in Fig. 1 a), significant difference existed in mating frequency ($F_{2, 57} = 9.11$, $P = 0.00$) and thrusting frequency ($F_{2, 41} = 17.97$, $P = 0.00$), but there was no significant difference in mating duration ($F_{2, 41} = 0.82$, $P = 0.45$) or in mating interval ($F_{2, 27} = 0.75$, $P = 0.48$). Multiple comparisons (LSD) showed the mating frequency of male was significantly higher in group I than in either group IV or group V ($P = 0.002$; $P = 0.00$, respectively), while between the two latter groups the difference was not significant ($P = 0.35$). The thrusting frequency was highest in group IV, followed by group V, and lowest in group I (group I and IV: $P = 0.00$; group I and V: $P = 0.00$; group IV and V: $P = 0.018$). For females (see in Fig. 1 b), there was no significant difference in mating frequency, mating duration or mating interval ($F_{2, 27} = 0.099$, $P = 0.91$; $F_{2, 36} = 0.52$, $P = 0.60$; $F_{2, 32} = 2.20$, $P = 0.13$, respectively).

Increasing the number of both male and female mating partners (see in Fig. 1 a and b respectively). Comparing with the group I, mating frequency of both male and female in group VI decreased significantly (male: $t = 2.508$, $df = 28$, $P = 0.018$; female: $t = 2.246$, $df = 28$, $P = 0.033$). While thrusting frequency in males increased significantly ($t = 7.284$, $df = 17.227$, $P = 0.00$), no difference in mating duration or mating interval was found in either male or female (male: $t = 1.326$, $df = 11.851$, $P = 0.21$; $t = 1.25$, $df = 23$, $P = 0.224$, female: $t = 1.575$, $df = 11.075$, $P = 0.143$; $t = 1.827$, $df = 18.511$, $P = 0.084$, respectively).

Influence of operation sex ratio and density on mating behaviour

Changing the number of mating partners also resulted in a change in operation sex ratio (R = male to female) and density (D = total number of voles in each treatment group) of voles. Values of R and D in each group were: group I: R = 1, D = 2; group II: R = 0.5, D = 3; group III: R = 0.33, D = 4; group IV: R = 2, D = 3; group V: R = 3, D = 4, and group VI: R = 1,
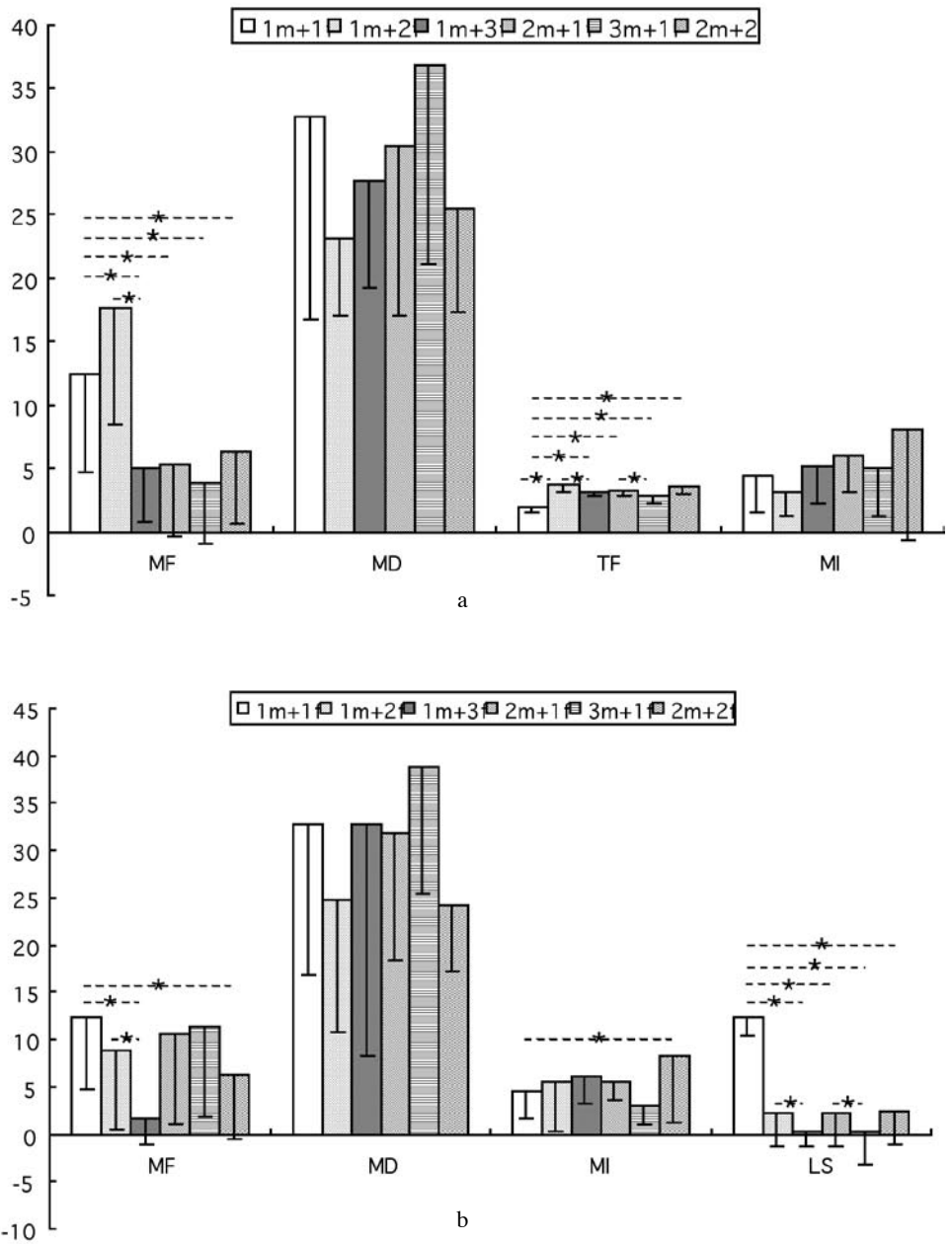
Fig. 1. Influence of increasing mating partners on copulatory behaviour and reproduction in Brandt's voles for (a) male and (b) female. MF – mating frequency, MD – mating duration, TF – thrusting frequency, MI – mating interval, LS – litter size. MI data unit is minute for convenience of drawing. *: $p < 0.05$.

$D = 4$. Male mating frequency was negatively correlated with R or D ($r_s^2 = -0.355$, $n = 100$, $P < 0.05$; $r_s^2 = -0.353$, $n = 100$, $P < 0.05$, respectively, Fig. 2 a and d). Negative correlations were also found between male thrusting frequency and R and between female mating

frequency and D ($r_s^2 = -0.281$, $n = 78$, $P < 0.05$; $r_s^2 = -0.422$, $n = 100$, $P < 0.05$, respectively, Fig. 2 b and f). Male mating duration and female mating times were both positively correlated with R ($r_s^2 = 0.317$, $n = 78$, $P < 0.05$; $r_s^2 = 0.486$, $n = 100$, $P < 0.05$, respectively, Fig. 2 c and e).
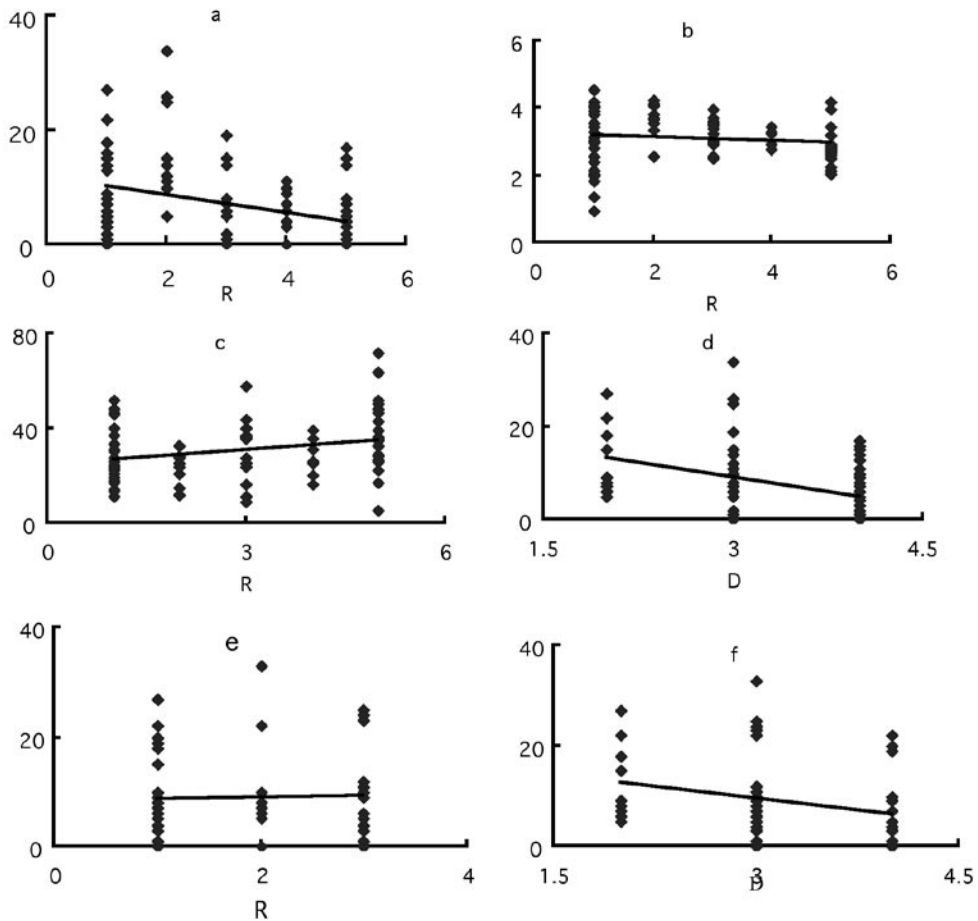

Fig. 2. Correlation of sex ratio and density with copulatory data in Brandt's voles. a: correlation of sex ratio with mating frequency of male voles; b: correlation of sex ratio with thrusting frequency of male voles; c: correlation of sex ratio with mating duration of male voles; d: correlation of density with mating frequency of male voles; e: correlation of sex ratio with mating frequency of female voles; f: correlation of density with mating frequency of male voles. MF – mating frequency, MD – mating duration, TF – thrusting frequency, R – operational sex ratio, D – density.

Effect of increasing number of mating partners on reproduction

Using mean litter size (LS) to represent the reproductive success of the vole, we found that, by increasing female partners only, there was a significant difference in LS (Kruskal-Wallis Test, $X^2 = 31.647$, $df = 2$, $P = 0.00$, Fig. 1 b). LS was highest in group I, followed by

group II and lowest in group III (Mann-Whitney test, between groups I and II: $U = 30.5$, $P = 0.001$; between groups I and III: $U = 8.0$, $P = 0.00$; between groups II and III: $U = 222.0$, $P = 0.010$). A significant difference was also found in LS in groups treated by increasing male partners ($F_{2, 27} = 5.912$, $P = 0.07$, Fig. 1 b). Multiple comparisons (LSD) showed that LS decreased significantly in group V compared with group I or group IV ($P = 0.02$; $P = 0.025$, respectably), but there was no significant difference between group I and group IV ($P = 0.338$). LS in group VI was much lower than in group I ($U = 16.0$, $P = 0.002$, Fig. 1 b). Pregnancy rate was much lower when a female mated five times or less than when a female mated more than five times (Chi-square test, $X^2 = 27$, $df = 1$, $P = 0.00$, see in Fig. 3).
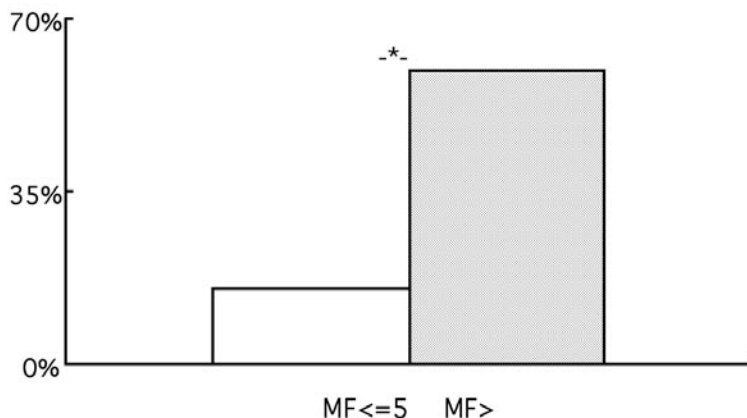

Fig. 3. Correlation of mating frequency and pregnancy rate in female Brandt's voles. MF – mating frequency. *: $p < 0.05$.

Discussion

Increasing the number of mating partners can change the operational sex ratio and density, which, in turn, can influence the mating behaviour of animals. This study shows that the operational sex ratio had a positive relationship with the mating opportunity of female Brandt's vole, while it had a negative relationship with the mating opportunity of male Brandt's vole. Density had a negative effect on mating opportunity for both males and females. Compared with the control group 1 ♂ + 1 ♀, when only increasing female partners, the OSR and density had different effect on the mating opportunity of male voles, while it had same effect on the mating opportunity of female voles. The OSR' s effect on the mating opportunity of male voles overran the density's effect in group 1 ♂ + 2 ♀♀, while it was reverse in group 1 ♂ + 3 ♀♀. When only increasing male partners, the OSR and density had different effects on the mating opportunity of female voles, while it had the same effect on the mating opportunity of male voles, and the effect of OSR and density on the mating opportunity of female voles counteracted. When increasing male and female partners at the same time, there only existed the density effect. Thus, higher density seemed to intensify mating interference. Thus our results support the idea of "competitively reproductive interference" (Z h a n g 2000). Also we found the mating duration of male voles had a positive relationship with the OSR. Maybe it represented a kind of mating compensation.

As stated above, the operational sex ratio had a positive relationship with the mating opportunity of female Brandt's vole, while it had a negative relationship with the mating opportunity of male Brandt's vole. This means, whether male or female voles, they all incline to mate with more different mates. This is very meaningful to voles. It not only increases the fitness of male voles, but also guarantees that the female selects the best sperm for pregnancy, also avoiding infanticide at the same time. Actually in many animals, it is true that both male and females exhibit extra-pair copulation behaviour, no matter what mating system the animal has (B i r k h e a d 1987). In Brandt's vole, field studies showed they had a family group in which one or several mature males lived with several mature females and their offspring but the adult ratio was more females than males during the breeding season (Z h a n g & Z h o n g 1981, W a n et al. 1998). This may indicate that the mating system of Brandt's vole is polygamy, and a previous laboratory study also support this (Z h a n g & Z h a n g 2003). However, we are unsure whether there exists extra-pair copulation behaviour.

Many female mammals mate multiple times before successful insemination as they need the stimulation to induce ovulation (H u n t e r et al. 1993, G i n s b e r g & H u c k 1998); these animals cannot ovulate spontaneously. Brandt's vole is one such animal (Research group I in Institute of Zoology, Chinese Academy of Sciences 1978). Our study has demonstrated that fewer than five mating times would result in a much lower pregnancy rate, and the litter size decreased dramatically when the number of mating partners increased. Hence, when density increased, the mating opportunity would decline due to mating interference, which, in turn, would influence the reproductive success. We thus conjecture that mating interference is highly likely a behavioural mechanism in regulating natural populations in this species.

Compared with the field, the laboratory environment has several limitations: the familiar time between the animals is too short, the space is too crowded and the environment is too simple. This leads to extensive interference between animals and may change the actual reproductive form of female voles. As suggested by M a p p e s et al. (1998), the most parsimonious explanation for breeding suppression in laboratory studies is that it may be a methodological or laboratory artifact. A previous study showed that the mean litter size of Brandt's vole in the field is 8 – 10 (W a n et al. 1998), while in our study, the mean litter size of each group all decreased severely compared with the field result. Thus, laboratory artifacts may be a possible reason leading to the decreased litter size. Moreover, as showed in other studies, many effects of pheromones can influence the mating behaviour of mice, such as the Coolidge effect and Whitten effect. In Brandt's vole, there have been no such studies. Therefore, we need to carry out more studies, especially field studies in the future to compare the results with those from the laboratory before our method can be applied effectively in controlling pest rodent populations.

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