Tactical reduction of copulatory competition: effects of male urine odour on maturation rates of Brandt’s voles, *Lasiopodomys brandtii*

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**Abstract.** Mammals’ urine contains chemosignals known to affect the sexual behaviour and reproductive physiology of both male and female recipient. Here we demonstrated that male urine can also elicit physiological changes on sexual maturation rates in the Brandt’s voles. In the first experiment we showed that urine from breeding adult male anticipated the puberty on female voles and at opposite delayed puberty on males. The results from the second and third experiments showed a strong agonistic behaviour between male Brandt’s voles compared to that of females. The introduction into social groups of an adult male, but not an adult female, increased the agonistic responses of the resident male. The results suggest a high rate of competition for mating in the male. Therefore, we conclude that a breeding adult male uses chemosignals to differently affect the sexual maturation of pre-pubertal conspecifics as a tactic for reducing competitive mating pressure. Little or no competitive pressure for copulation was seen in the oestrous female, and its urine had no effect on the maturation rate of young Brandt’s voles.

**Key words:** odour of urine, maturation rate, agonistic, non-agonistic, Brandt’s vole

**Introduction**

Chemosignals are known to influence reproductive behaviour and neuroendocrine function in many social mammals. They are used, in effect, as a tactic for obtaining more copulatory opportunities. Olfactory cues have most effects on interactions between conspecifics or between sexual partners. Physiological changes induced by sexual chemosignals are frequently stereotyped responses (Keverne et al. 1986). For example, in the mouse (Vandenberg 1969), prairie vole (*Microtus ochrogaster*) (Carter et al. 1980) and hamster (*Phodopus campbelli*) (Reasner & Johnston 1988), experiments have been used to demonstrate that the odour of breeding animals’ urine can accelerate growth and puberty in juveniles that have never been in contact with adult males. In hamsters, chemosignals from female vaginal secretions induce an increase of testosterone in the young males (Pfeiffer & Johnston 1994). Naive male mice also reflexively release luteinizing hormone and testosterone pulses after encountering a female or her urinary pheromones (Macrides 1975, Maruniak & Bronson 1976). In addition, some molecules certified can alter the maturation rate of young. E.g. 2, 5-dimethylpyrazine and 2-heptanone can delay puberty of young mouse and 6-hydroxy-6-methyl-3-heptanone can accelerate puberty (Dulac & Torello 2003). Chemosignals may also induce stereotyped behavioural responses. For example, in the hamster, vaginal secretions can induce young males to copulate with anaesthetized surrogates (Murphy 1973, Macrides et al. 1977, 1984). In a variety of species, for example rats (*Rattus rattus*), mice and dogs (*Canis lupus*), chemical signals serve as sexual attractants and induce sexual arousal, pre-copulatory postures and mating behaviour.

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but these responses are expressed preferentially by breeding adults (Carr 1965, Doby & Dunbar 1974, Hayashi & Kimura 1974). Equivalent responses of young Brandt’s voles (Lasiopodomys brandtii) to olfactory cues have not yet been reported.

The maintenance of dominance hierarchies is an important component of the behaviour of social animals (Piper 1997). Dominant individuals play an important role in stabilizing the social system (Fedigan 1983). For example, a dominance hierarchy can reduce the degree of fighting among individuals to the advantage of both dominant and subordinate individuals. The result is that dominant individuals have priority access to resources, such as shelter, food, water, nest sites and mates (Wiley 1991). There may be an increase in the longevity of dominant individuals, which also increases their opportunities for copulation. Dominant individuals must adopt tactics to maintain their social status especially if there is strong competition for copulations. For example, male field voles (Microtus agrestis) reduce competition by marking copulatory domains (Nelson 1995), and female Eurasian dotterels (Charadrius morinellus) obtain more copulations through intra-sexual fighting (Owens 1994). At present there is little information on the tactics used by Brandt’s voles to reduce copulatory competition.

Brandt’s voles are social mammals that mainly inhabit the grasslands of Inner Mongolia in China, as well as Mongolia and the region around Beigaer Lake in Russia (Shi 1988). Population densities vary greatly between years, with outbreaks occurring once every 5–7 years (Zhang 2001). In stable social groups there appears to be little competitive behaviour for copulation, although there is a strict social hierarchy (Chen & Shi 2003). Therefore, we designed a series of experiments to understand the mechanisms used by Brandt’s voles for reducing competition for copulation.

Firstly, we tested whether adult male Brandt’s voles could potentially increase their copulatory opportunities by releasing chemosignals to induce physiological changes in young males and females. Then we assessed the strength of copulatory competition between adult males compared to oestrus females. Experiment 1 was designed to measure the rate of maturation and growth of young Brandt’s voles exposed to the odour of urine from breeding adults. Experiment 2, we observed pairwise interactions to determine whether breeding male Brandt’s voles have a higher frequency of competitive behaviours than the oestrus females. Finally, in Experiment 3, we observed behavioural interactions following the placement of a breeding Brandt’s vole with a male and female pair from a different social group to determine whether male Brandt’s voles have more competitive pressure for copulation than females.

Material and Methods

Experiment 1

Animals

Founders of a laboratory colony of Brandt’s voles at the China Agriculture University were sourced from Inner Mongolia. We used 80 laboratory-bred Brandt’s voles: young Brandt’s voles (25 days old) (n = 30 male, n = 30 female), adult male Brandt’s voles in breeding condition (enlarged testes and obvious mating behaviour) (n = 10), oestrus female Brandt’s voles (obvious perforate vagina) (n =10). The Brandt’s voles were held in glass boxes measuring 40cm×20cm×30cm (temperature 25±1°C; photoperiod 14L:10D), with ad libitum food (cabbage and cornmeal) and water.
Experimental protocol
We conduct the experiment at 9:00 AM in the morning. Urine was collected from individually-
caged, adult Brandt’s voles in breeding condition. Because the volatile elements of urine are
likely to be important, we conducted Experiment 1 immediately the urine was collected. The
filter paper with adsorbing the testing urine hung over the glass boxes. The young voles can’t
touch the stimuli with the nose.

The 25-day-old Brandt’s voles were selected to have similar weights (16±1g, to
eliminate size bias) and, for each sex, divided into 3 groups of 10. Individuals from Group
I were exposed to a filter paper soaked with urine of adult male Brandt’s voles in breeding
condition, individuals from Group II were exposed to a filter paper soaked with urine of
adult female Brandt’s voles in oestrus, and individuals from Group III were controls.

During the period of exposure to the urine, all groups of young Brandt’s vole were
isolated from each other so that each group was affected by only one kind of urine odour. To
avoid depletion of volatile elements, we regularly replaced the filter papers in every group
by fresh filter paper soaked with urine. The onset of sexual maturity in male Brandt’s vole
is marked with enlarged testes and mating behaviour. Also young male Brandt’s voles with
enlarged testes were tested for mating behavior with estrous females for ten minutes every
day. Sexual maturity in females is marked with the appearance of a perforate vagina. We
recorded the time for each animal to reach sexual maturity.

Experiment 2

Animals
Brandt’s voles were collected from grassland typical of Taipusi Banner (county) in Inner
Mongolia and bred in the laboratory at the China Agriculture University. We selected adult
male Brandt’s voles in breeding condition (enlarged testes) and oestrus females. Their average
weight was 38.7±1.1g and they were 7–8 months old.

Each vole was housed separately in a 28cm×17cm×15cm plastic box provided with
hay and cotton, supplied with ad libid. food and water, and held under the same ambient
conditions (temperature 25±1°C; photoperiod 14L:10D).

Experimental protocol
The adult male Brandt’s voles were allocated to 23 pairs and the females were allocated to 17
pairs, each pair consisting of unrelated, similar-sized (< 3g difference in weight) individuals.
Observations of the behaviour between the two individuals of each pair were conducted
in a separate room using a transparent 90cm×30cm×30cm cuboid. A plastic 30cm×30cm
baffle divided the space into two 45cm×30cm×30cm sections. One animal was placed at one
end of the cuboid and the other member of the pair at the opposite end. Initially a round
doorsway (10cm diameter) at the bottom of the baffle was closed. Then, after opening the
doorsway, the number of occurrences of each type of behaviour of the two Brandt’s voles
was recorded for 30 minutes. Their behaviour was classified as follows: agonistic behaviour
(an individual chases, attacks and fights the other individual), non-agonistic behaviour (an
individual sniffs or smells a certain position). All these behavioural patterns followed the
definitions of Francis (1988), Fang (1994) and Ekland (1996). The cuboid was
cleaned thoroughly and deodorized using 75% ethanol solution (v/v) after the recording
session for each pair.
Experiment 3

Animals
Brandt’s voles were collected from grassland typical of Taipusi Banner in Inner Mongolia and bred in the laboratory at the China Agriculture University. We selected 40 adult pairs (1♀+1♂; <3g difference in weight), with the members of each pair coming from the same social group. In addition, we selected 20 breeding male and 20 oestrus female Brandt’s voles as unacquainted interveners, each being a similar-sized (<3g difference in weight) individual sourced from outside the social groups of the pairs. Their average weight was 39.7±1.2g. All voles were housed in the same conditions as for Experiment 2.

Experimental protocol
The environment for observation was the same as for Experiment 2. One pair was placed at one end of the cuboid and the unacquainted individual at the opposite end. Initially the round doorway (10cm diameter) at the bottom of the baffle was closed. Then, after opening the doorway, the number of occurrences of each type of behaviour was recorded for 30 minutes. Types of behaviour were classified as follows: agonistic behaviour (an individual chases, attacks and fights the other individual of the same sex), non-agonistic behaviour (an individual sniffs or smells a pubic position, and mating) and investigative behaviour (an individual sniffs or smells a pubic position of an unacquainted animal). All these behavioural types followed the definitions of Francis (1988), Fang (1994) and Ekland (1996).

Data analysis
Data were analysed using the SPPS software package (10.0). Distributions of all variables were tested for normality using the Kolmogorov-Smirnov test. Group differences in time to maturation were analysed by a one-way analysis of variance (ANOVA). The same testing one-way ANOVA was also used to analyse behavioural difference between males and females. Finally, Pearson’s correlation was used to compare the relative frequency of two behaviours. Statistical significance was determined at P<0.05.

Results
In Experiment 1, the time to sexual maturity of young male Brandt’s voles in Group I, which were exposed to the urine of adult male Brandt’s voles in breeding condition, was significantly longer than in other groups (F(2,27) = 27.187, P < 0.05) (Fig. 1A). There was no difference between the other two groups of young males (F(1,18) = 0.112, P > 0.05) (Fig. 1A).

The time to sexual maturity of young female Brandt’s voles in Group I (exposed to the urine of adult male Brandt’s voles in breeding condition) was significantly shorter than in other groups (F(2,27) = 15.577, P < 0.05) (Fig. 1B). No difference was found between the other two groups of young females (F(1,18) = 0.308, P > 0.05) (Fig. 1B).

In Experiment 2, the frequency of agonistic behaviour between male Brandt’s voles was significantly higher than females (F(1,36) = 99.889, P < 0.05) (Fig. 2). There was no detectable difference between male pairs and female pairs in non-agonistic behaviour (F(1,36) = 3.358, P > 0.05) (Fig. 2).

In Experiment 3, when an unacquainted (not the same social group) breeding male Brandt’s vole was placed with a pair (1♀+1♂ from the same social group) the agonistic
behaviour (frequency showed in the Fig. 3) between the male Brandt’s voles had a higher frequency than was recorded between paired males in Experiment 2 ($F_{1,40} = 6.582, P < 0.05$), and the frequency of non-agonistic behaviour (frequency showed in the Fig. 3) between the
male Brandt’s voles was changed relative to that between paired males in Experiment 2 ($F_{(1,41)} = 6.144, P > 0.05$) (Fig. 4). However, when an unacquainted (not the same social group) oestrus female Brandt’s vole was placed with a pair ($1♀+1♂$ from the same social group), the frequency of agonistic and non-agonistic behaviour between female Brandt’s voles was no different from that recorded between paired females in Experiment 2 ($F_{(1,33)} = 0.456, P > 0.05; F_{(1,33)} = 0.144, P > 0.05$) (Fig. 4).

Finally, in Experiment 3 the frequency of female investigative behaviour to an unacquainted (not from the same social group) breeding male Brandt’s vole was positively correlated with agonistic behaviour between male Brandt’s voles ($r = 0.771, P < 0.05$) (Fig. 5A). In contrast, the frequency of male investigative behaviour of an unacquainted (not from the same social group) oestrus female Brandt’s vole was not correlated with the frequency of agonistic behaviour between female Brandt’s voles ($r = 0.128, P > 0.05$) (Fig. 5B).

Discussion

Young female Brandt’s voles in Group I reached sexual maturity at least 13 days earlier than both the control group and the group that was exposed to urine from the oestrus female.
(Fig. 1B). The results for Group I are consistent with observations of many mammal species in which male odour can accelerate sexual maturation in young females. Examples include microtine rodents (Richmond & Conaway 1969, Richmond & Stehn 1976), the mouse (Vandenbergh 1969), prairie vole (Microtus ochrogaster) (Carter et al. 1980), hamster (Phodopus campbelli) (Reasner & Johnston 1988), cow (Bos taurus) (Izard & Vandenbergh 1988) and the mouse (Dulac & Torello 2003).

Young male Brandt’s voles in Group I reached sexual maturity at least 19 days later than both the control group and the group that was exposed to urine from the oestrus female (Fig. 1A). This affect has also been demonstrated in other mammalian species. For example, in the mouse (Maruniak & Bronson 1976) and hamster (Phodopus campbelli) (Pfeiffer & Johnston 1994) males exhibit a reflexive increase of testosterone during their first exposure to female urine or vaginal secretions. To these observations, we can add our results that the odour of urine from breeding male Brandt’s voles can delay significantly the onset of sexual maturity in young male Brandt’s voles. In addition, some reviews on Pheromonal communication in vertebrates revealed the reason of urine effect on the maturation rate of young (Brennan & Zufall 2006, Stowers & Martin 2005).

The results from Experiment 1 demonstrate that odour from the urine of adult male Brandt’s voles alters the maturation rates of young males and females. The outcome is non-synchronous recruitment of male and female Brandt’s voles to the breeding population and, therefore, for adult males a potential reduction in competition for copulatory opportunities. Experiments 2 and 3 demonstrated that copulatory competition is important for male Brandt’s voles but not females.

Intra-sexual competition between animals in the breeding condition is often expressed through fighting for opportunities to mate (Owens 1994, Nikitopoulos & Arnhem 2004), i.e. the relative frequency of agonistic and non-agonistic behaviour is an index of competition for copulation. Of course, the mating system of Bandit’s vole could also be adapted to deal with this competition. But assessments of the mating system are contradictory; for example it was considered polygynous by Xie et al. (1994) but promiscuous by Yin & Fang (1998). Therefore we used observations of the agonistic and non-agonistic behaviour of Brandt’s vole in Experiments 2 and 3 to understand why urine odour from adult male Brandt’s vole changed the maturation rate of young voles, whereas urine odour from oestrus females had no effect.

The results from Experiment 2 demonstrated that agonistic behaviour between male Brandt’s voles was stronger than females, whereas there was no difference in non-agonistic behaviour (Fig. 2). In Experiment 3, we found that the frequency of agonistic behaviour between male Brandt’s voles was enhanced and non-agonistic behaviour was reduced by introducing an unacquainted male Brandt’s vole in breeding condition into a social group (1♀+1♂). In contrast, the introduction of an unacquainted oestrus female into a social group had no effect on the frequency of agonistic and non-agonistic behaviour (Fig. 4), i.e. unacquainted oestrus female Brandt’s voles were accepted more readily than unacquainted breeding male Brandt’s voles by the social group. Therefore, we conclude that there is more copulatory competition between male Brandt’s voles than between females.

Further, the agonistic behaviour between male Brandt’s voles was positively correlated with the frequency of female investigative behaviour (Fig. 5A), but male investigative behaviour of an unacquainted female resulted in no change in female agonistic behaviour (Fig. 5B). In the field, the recruits are accepted by social groups continuously during the
breeding season (Shi & Hai 1999). The results of Experiment 3 show that female investigative behaviour to unacquainted breeding male Brandt’s voles would add pressure to the males in a social group.

The behavioural data show that breeding male Brandt’s vole face strong copulatory competition within their social group. Their tactical response is to release chemosignals that alter the maturation rates of male and female Brandt’s voles resulting in non-synchronous recruitment of males and females to the breeding population and therefore enhanced mating opportunities for adult males. Thus, the copulatory competition of brandt’s vole was reduced by release chemosignals, which was propitious to its social stabilization.

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Literature


