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# Scent, social status, and reproductive condition in rat-like hamsters (*Cricetulus triton*)

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**Abstract**

This study investigated the interrelations among scent glands, social status, and reproductive conditions in male rat-like hamsters (*Cricetulus triton*), along with differential attraction of estrous females to conspecific males with different social status and reproductive condition. First, there were positive correlations between testes weights and flank glands and midventral glands. Second, castrated males were dominated by both intact males and castrated males treated with testosterone. Third, estrous females were less attracted to the scents from flank glands and midventral glands of castrated males. Moreover, dominant males had heavier testes and higher levels of circulating testosterone, and estrous females were more attracted to dominant males' scents. Our results indicated that estrous females were able to discriminate between the odors of two strange males with differences in reproductive conditions or social status. 

*Keywords:* Social status; Reproductive condition; Scent; Rat-like hamsters (*Cricetulus triton*)

## 1. Introduction

The growth of some scent glands in male mammals is largely under the control of androgen [4,8,20]. Size of these scent glands is positively correlated with testes size and dominance hierarchy in male mammals [4,10,11,17,19,20]. At the same time, the hormonal status of animals has been found in some mammal species to be related to the animal's social rank [14,18,21,22]. Therefore, these scent glands are also related to social rank as well as hormonal status [10,11,15,17]. The dominant males with high testicular activity favor a mate's reproductive success [12,13]. It is very important for females to discriminate the quality of potential mates to insure their successful reproduction.

Females could base their choice among different-ranking males on one of two different mechanisms: (1) through individual association and recognition, females can identify every male in a social group, (2) on the basis of signals that are influenced by endocrine factors, females could choose between males of high and low ranks [16]. Rat-like hamsters (*Cricetulus triton*) are an asocial, promiscuous species living in the farmland in North China [24,26]. Males have a pair of flank glands and a midventral gland, display high recruitment, and are dispersal-prone; females are philopatric and have no stable mating association [26–28]. Therefore, the aim of this study was to investigate if there is a positive correlation among scent glands, endocrine condition, and social status in male rat-like hamsters and if estrous females are able to recognize strange males with differences in endocrine and social status on the basis of scent cues.

## 2. Method

### 2.1. Subjects

About 150 male and 50 female adult rat-like hamsters (more than 80 g of body weight) utilized in this study were captured in livetraps made of wire mesh in the farmland of Hebei Province, North China, during spring and summer (breeding season) of 1996 to 1999. Fifty-four of these males (mean body weight ± S.D. = 139.41 ± 24.07 g) were used to determine the correlation between testes weight and scent gland weights. They were killed soon after capture,

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their carcasses were sealed individually in plastic bags and kept at −20°C until postmortem examination. The flank glands and midventral glands are obviously thicker than surrounding skin. We first shaved the fur on the flank glands or midventral glands, then cut off the glands along their outline on the inner skin. The following data were collected for each animal: whole body weight (±0.1 g), mean weight of a pair of testes (0.1 mg), mean weight of a pair of flank glands (0.1 mg), and weight of midventral gland (0.1 mg). The partial correlation coefficient between testes and scent gland weights using whole body weight as covariant was calculated by SPSS (Version 6.0) software in computer.

Thirty male and 30 female rat-like hamsters utilized in successive behavioral experiments were selected from a larger number of captured hamsters. In each male–female pair, the male and female came from different areas, which were separated by rivers, highways, or railway dams, and among which the distances were more than 1 km (males' maximum activity range is 1 km) [23]. We can therefore assume that the paired hamsters were unfamiliar with each other. Their breeding condition was determined upon capture. Those weighing more than 100g were assumed to have had sexual experience [24]. Scrotal males and females with perforate vaginas were classified as potential breeders and were used in the experiment. The estrous cycles of females were determined by extravaginal examination and behavioral receptivity to males.

The hamsters were maintained in a reversed light: dark cycle of 14:10 (lights on at 1700 h) at approximately 20°C for at least 2 weeks prior to surgery and behavioral testing and were housed individually in wire mesh cages ($40 \times 25 \times 15$ cm$^3$) containing wood shavings and cotton nesting materials with rat chow and water provided continuously. Twenty males were castrated; 10 of these were implanted with a testosterone capsule at the time of castration. The treated males were used in behavioral tests 3 weeks after surgery. Two males of approximately the same body weight (10%) were assigned to pairs and were kept in a cage for 14 consecutive days to establish the differences in scents and endocrine levels induced by dominant–subordinate relations. After intact females had been in captivity for 5 weeks, they were in estrus judged by vaginal secretion detection and behavioral responses to males, and were used in behavioral tests.

### 2.2. Surgical procedure

The males were anesthetized with sodium pentobarbital (60 mg/kg). Castration of the males was performed through bilateral incisions in the scrotum. Testosterone capsules were constructed from 15 mm of Silastic tubing (China Medical, o.d. 2.70 mm, i.d. 2.26 mm), which was packed with 10-mm lengths of crystalline testosterone (Sigma, St. Louis) and sealed with 2.5-mm lengths of Medical Adhesive Silicone Type A (Dow Corning) at both ends. The capsules were implanted subcutaneously in the dorsal area of waist via a small incision at the time of castration. The wound was closed with sterile sutures and treated with 5% tincture of iodine and crystalline sulfanilamide.

### 2.3. Measurement of testosterone level

On the day after the completion of the behavioral testing in other experiments, scent stimulus males were anesthetized with sodium pentobarbital and sacrificed, and trunk blood was obtained. Blood was centrifuged at 4000 rpm and serum was collected and stored at −20°C until radioimmunoassay. Radioimmunoassays were run using the reported methods [25] in the Laboratory of Reproduction Biology, Institute of Zoology, Chinese Academy of Sciences. $^3$H-Testosterone was from the Shanghai Nuclear Institute of Chinese Academy of Sciences, the antibody was from the Institute of Zoology, Chinese Academy of Sciences, and the standard testosterone was from Organ, Holland. The detectable range of the assay was 0.25–8.0 ng/ml. Intra-assay coefficient of variation was 7.25%.

### 2.4. Behavioral testing

#### 2.4.1. Quantification of agonistic behavior

All staged dyadic encounters were conducted in an observing room under dim, red illumination during the hamsters' dark cycle. All rat-like hamsters were tested once. Paired encounters took place in a clear glass box ($105 \times 35 \times 40$ cm$^3$), which was partitioned to three equal parts by two removable opaque Plexiglas plates. Two male hamsters from different areas with similar weight (within 10% difference) were placed respectively into two partitions at each end of the box as a pair. Following a 5-min acclimatization period, the two opaque partitions were removed and the hamsters were allowed to interact. The frequency of several behavior patterns was continuously recorded for 5 min by hand on a data sheet with a precalibrated time scale (10 s). Behavior pattern having a duration of 10 s or less was treated as one unit; if the duration was greater than 10 s but less than 20 s, the act was considered two units, and so on. The time was measured with a stopwatch [9].

The glass box was thoroughly cleaned between trials with hot 1 M sodium hydroxide solution followed by both water and ethanol.

The following definition was used to describe social behaviors: *attack* included biting, chasing, and sideways postures; *defense* included fleeing and upright postures; *marking* was defined as hamsters arching their backs out towards a wall and vigorously rubbing their flank glands against the wall. Subordinate hamsters deferred to a dominant attacker by fleeing, cowering, or exhibiting upright postures [26]. We analyzed behavioral differences by Wilcoxon matched pairs tests.

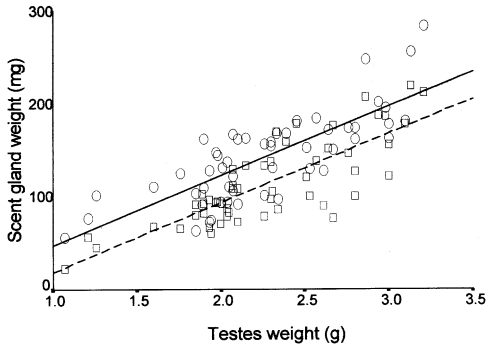


Fig. 1. Linear regression between scent gland weight (mg) and testes weight (g). Legend: - - -, □: midventral gland, $r = .8057$, $P < .001$. –, ○: flank gland, $r = .7824$, $P < .001$.

*2.4.2. Odor testing procedure*

We tested hamsters in their home cages under red light in a room that was separated from the colony. Scents were presented by placing a glass plate 18 cm in length and 6 cm in breadth on the bottom of a hamster's home cage. This glass plate was divided into three equal sections: one end section contained a stimulus odor from one group of males, the other end section contained a stimulus odor from the same source from the other group of males, and the middle section remained clean. We recorded the time spent investigating the scented sections of the glass plate during a 5-min test with two stopwatches. Criteria for investigation of a scent were that a hamster obviously licked or sniffed a stimulus scent and /or its nose was above the designated end of the plate and within 1 cm of the surface. The test was initiated when the experimental hamster performed its first investigation. Placement of stimulus odors on the left or right end of the plate was at random. After the experiment, we cleaned the glass plates with laboratory glassware cleaner, rinsed them in tap water followed by ethanol, and then dried them with hot air flow. We wore disposable plastic gloves when handling the glass plates so as not to transfer our scent to the plates. Wilcoxon matched pairs tests were used in this study to test for significant differences between the total time hamsters spent investigating each stimulus pair. Significant differences were accepted at $P < .05$ (two-tailed test).

In every test, we selected 10 estrous subjects from 30 females. We tested these subjects for their preferences for male versus female odors using flank gland and midventral gland secretions. At least 4 days intervened between use of an individual in two tests.

*2.4.3. Odor collection*

We shaved the fur around the donor hamsters' flank glands and midventral glands, so the scent could easily be obtained directly from the gland surface with a cotton swab. The cotton swab was rubbed against the gland surface for about 10 s and then rubbed against one end of the glass plate for 10s. Unless otherwise noted, all scents were collected fresh for each trial from scent donor hamsters by rubbing a cotton swab against its flank gland and midventral gland surfaces.

## 3. Results and discussion

### *3.1. Scent glands and testes*

There was a significant, positive correlation between testes weight (average of both testes) and both flank gland weight (average of both glands) ($r = .78$, $n = 54$, $P < .0001$) and midventral gland weight ($r = .81$, $n = 54$, $P < .0001$) (Fig. 1). Since the weights of both testes and two scent glands correlate significantly with body weight (testes weight and body weight, $r = .53$, $n = 54$, $P < .0001$; flank gland weight and body weight, $r = .55$, $n = 54$, $P < .0001$; midventral gland weight and body weight, $r = .44$, $n = 54$, $P < .001$), the partial correlation coefficient was calculated using body weight as the covariant. The testes weight was found to correlate significantly with both flank gland weight ($r_p = .75$, $n = 54$, $P < .001$) and midventral gland weight ($r_p = .69$, $n = 54$, $P < .0001$).

The results suggest that males with greater activity of their testes, irrespective of body size, can maintain greater flank glands and midventral glands. This indicates that these two scent glands in rat-like hamsters can be significantly affected by hormones. Therefore, the two scent glands may be related to social status in rat-like hamsters.

### *3.2. Effects of testosterone on social status and scent glands*

Castrated males developed lighter flank glands and midventral glands than intact males; testosterone replacement

Table 1
Effects of gonadectomy and testosterone replacement on scent gland weight (mean ± S.D.)

| | Castrated | Intact | Castrated with testosterone replacement |
|---|---|---|---|
| Sample size | 10 | 10 | 10 |
| Body weight (g) | 155.3 ± 11.2 | 148.9 ± 9.1 | 150.1 ± 12.7 |
| Testosterone level (ng/ml) | 0.479 ± 0.113 | 3.215 ± 1.027** | 3.510 ± 0.868** |
| Flank gland (mg) | 80.5 ± 20.0 | 136.5 ± 31.1** | 146.3 ± 47.5** |
| Midventral gland (mg) | 61.3 ± 16.6 | 84.4 ± 7.2* | 96.53 ± 20.06** |

* $P < .05$ indicate a significant difference between intact and castrated males using independent samples $t$ test.

** $P < .01$ indicate a significant difference between intact or testosterone-treated males and castrated males using independent samples $t$ test.

restored the flank gland and midventral gland weights of castrated males (Table 1). In paired encounters between intact and castrated males, intact males displayed more attack, less defense, and more flank marking (Fig. 2a). Testosterone replacement restored significantly the dominance of castrated males as shown by differences in attack, defense, and flank marking (Fig. 2b). Estrus females were significantly less attracted to the glandular secretions from castrated males than to those from intact males and castrated males treated by testosterone (Fig. 3).

The above results indicate that serum androgen can regulate social status, the size of the two scent glands, flank gland marking, and differential attractiveness of the two scent glands' secretions to estrus females. Without previous social experience with males, estrus female rat-like hamsters can discriminate between the scents of males having a large difference in hormonal levels and consequent difference in the level of dominance exhibited by the male and they prefer the above male with higher testosterone level.

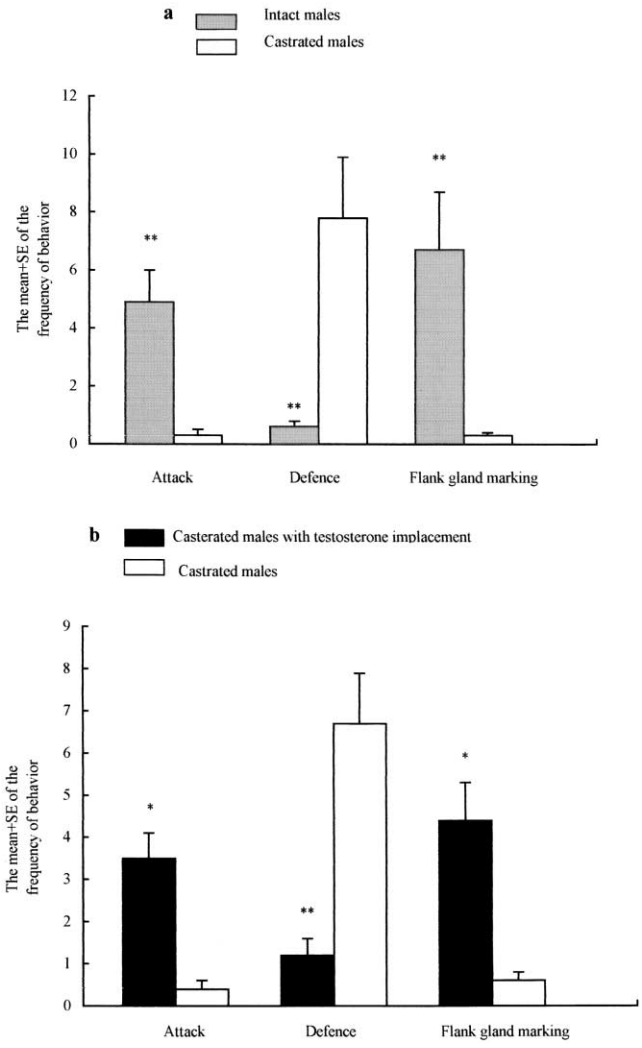


Fig. 2. Behavior during staged dyadic encounters of male rat-like hamsters with differences in reproductive condition. * $P<.05$ and ** $P<.01$, using Wilcoxon matched pairs tests.

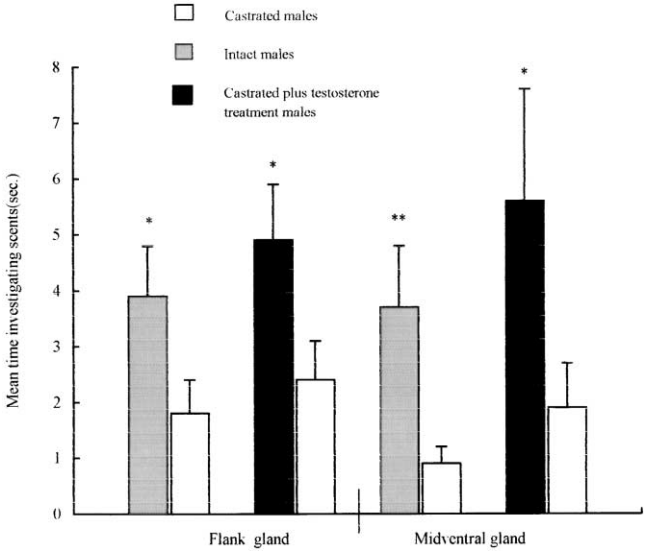


Fig. 3. Mean time ± S.D. (s) that females investigated scents from scent glands of males in different testosterone conditions. * $P<.05$, using Wilcoxon matched pairs tests.

### 3.3. Effects of social status on testes and scent glands

In paired encounters between two males, the dominant one had significantly heavier testes, flank glands, midventral glands, and higher level of testosterone (Table 2). This indicates that male's social status is closely related to the size of scent glands and testosterone level.

Estrus females spent more time investigating the secretions from flank glands and midventral glands of dominant males than those of subordinate (Fig. 4). This indicates that the estrous females can discriminate between dominant and submissive males by their scents without previously social association.

### 3.4. General discussion

This study has shown that in rat-like hamsters, there was a significant correlation between dominance levels, the size of two scent glands as well as the frequency of flank gland marking and testis size and testosterone level. In addition, estrous females could choose between different males on the basis of olfactory cues associated with physiological and social status without previous social association with the males.

Many studies have revealed the positive correlation between scent gland size, testis size, and dominance status in male rodents [1,3,4,10,11,17,19,20]. Marking and development of the specialized skin glands are often under the control of androgens [4,8,20]. At the same time, the hormonal status of animals has been found in male mammals to be related to their social rank [14,18,21,22]. In addition, the

Table 2
Differences in behavior, testes, and flank glands between dominant and submissive male rat-like hamsters (*C. triton*)

| Results of encounter | Frequency in staged dyadic encounters: Attack, mean ± S.E. | Defense, mean ± S.E. | Flank gland marking, mean ± S.E. | Body weight (g), mean ± S.D. | Testes weight (g), mean ± S.D. | Flank gland weight (mg), mean ± S.D. | Midventral gland weight (mg), mean ± S.D. | Testosterone level (ng/ml), mean ± S.D. |
|---|---|---|---|---|---|---|---|---|
| Dominance | 11.2 ± 1.8 | 0.1 ± 0.1 | 8.5 ± 1.7 | 142.3 ± 26.7 | 2.45 ± 0.98 | 151.2 ± 26.8 | 128.8 ± 32.7 | 3.97 ± 0.267 |
| Submission | 0.2 ± 0.1 | 13.4 ± 1.5 | 0.1 ± 0.1 | 139.8 ± 16.5 | 1.77 ± 0.47 | 107.9 ± 34.7 | 92.8 ± 30.7 | 2.781 ± 0.199 |
| *P* value | < .01, Wilcoxon test | < .01, Wilcoxon test | < .01, Wilcoxon test | > .05, $t = 0.95$ | < .01, $t = 3.36$ | < .01, $t = 4.06$ | < .05, $t = 3.10$ | < .05, $t = 2.93$ |
| *n* | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

size of scent glands and the frequency of marking with these specialized skin glands are also related to hormone-mediated dominance [10,11,15,17]. Male rat-like hamsters seem to be typical of this general pattern.

Dominance status in males is related to differential male reproduction. The majority of offspring in a population is sired by dominant males (e.g., *Peromyscus maniculatus*) [7]. Some explanations for the low fertility of subordinates have focused on the male's role in mating behavior in mammalian species. For example, some studies attributed this phenomenon to dominant males preventing subordinate males' access to receptive females (e.g., in laboratory rats and mice) and to reduced testicular activity in subordinate males (e.g., in laboratory mice, microtine rodents, and *Dicrostonyx groenlandicus*) [2,5,6]. Most female mammals make a greater parental investment than do males; hence, they should be particularly adapted to discriminate among potential partners

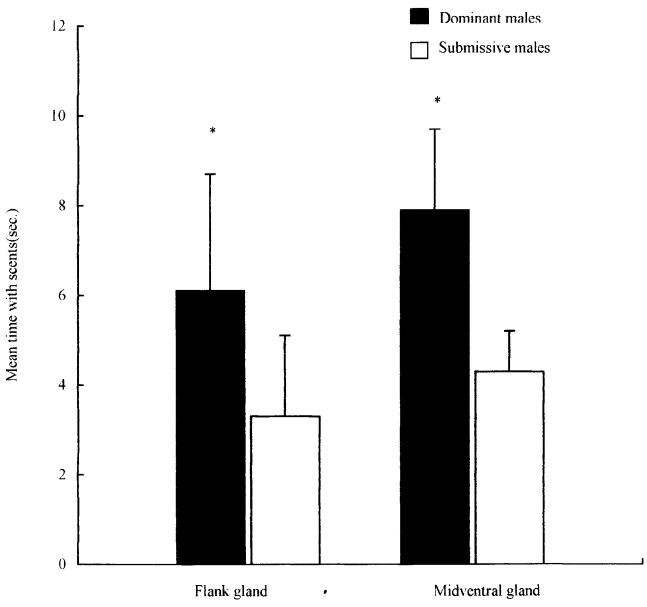


Fig. 4. Mean time ± S.D. (s) that females investigated scents from scent glands of dominant and submissive males. * $P < .05$, using Wilcoxon matched pairs tests.

along any dimension (e.g., social dominance, gonad, and endocrine condition) relevant to differential reproduction. For example, Huck and Bank [12,13] stressed the importance of the female's role in mating decisions and found that receptive female brown lemmings (*Lemmus trimucronatus*) spent more time and had more copulations with tethered dominant males than tethered subordinate males, and they preferred the body odor of dominant males to that of defeated males. Huck et al. [15] also found that estrus female golden hamsters (asocial species; *Mesocricetus auratus*) preferred dominant males to subordinate males. In conclusion, testicular activity and social status of males are very important for successful reproduction. Similarity, estrus female rat-like hamsters displayed a preference for the male's scents with higher levels in testosterone and dominance.

In solitary rat-like hamsters, females lack a long association with mates and provide almost all the parental effort during pregnancy and nursing [26]. So, it is more important for a solitary female than a female of a social species to choose a high-quality mate. There is the possibility for a solitary and promiscuous female rat-like hamster to meet more than one strange male [23]. Our results indicated that female rat-like hamsters were able to select high-quality mates through olfactory cues associated with male's endocrine condition and dominance status. Consequently, the high-quality males may increase the female's reproductive success such as litter size and offspring's survival. Scent signals play a very important role in the choice of male for female rat-like hamsters, although it needs further study in the field.

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