Physiology & Behavior 143 (2015) 90–96

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Hormones orchestrated pre- and post-copulatory sexual traits in male Mongolian gerbils

Wei Shen [a,b], Xue-Ying Zhang [b,*], Ding-Zhen Liu [a], De-Hua Wang [b]

[a] Ministry of Education, Key Laboratory of Biodiversity Science and Ecological Engineering, College of Life Sciences, Beijing Normal University, Beijing 100875, China
[b] State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China

HIGHLIGHTS

- Dominant gerbils had more and better quality of sperm than subordinate gerbils.
- Dominant gerbils had the same energy intake and RMR as subordinate gerbils.
- There was no trade-off between pre- and post-copulatory sexually selected traits.
- Aggression level and sperm quality increased with elevated T but reduced CORT.
- CORT and T orchestrated relationship between pre- and post-copulatory sexual traits.

ARTICLE INFO

Article history:
Received 18 December 2014
Received in revised form 16 February 2015
Accepted 24 February 2015
Available online 25 February 2015

Keywords:
Mongolian gerbil
Social status
Sperm quality
Corticosterone (CORT)
Testosterone (T)
Energy intake

ABSTRACT

Parker's sperm competition model predicts a negative relationship between pre-copulatory (social status) and post-copulatory (sperm quality and quantity) sexually selected traits, however, empirical studies have revealed considerable inconsistency in this relationship. We hypothesized that there was a trade-off between pre- and post-copulatory sexually selected traits, and hormones (corticosterone, CORT; testosterone, T) orchestrate this relationship. In this study, we measured energetic parameters in the dominant–subordinate Mongolian gerbils (*Meriones unguiculatus*), whose relationship was established under chronic social encounters in a neutral arena, and then tested the relationship between their social status and sperm quality and quantity. Our results showed that dominant males initiated attack sooner and displayed more aggression, self-grooming and locomotion behaviors in daily social encounters across seven consecutive days. Dominant gerbils also had more and better quality of sperm than that of subordinate males, yet showed no significant differences in energy intake and RMR in comparison with subordinate individuals. In addition, dominant males had higher concentrations of serum T than subordinate males, whereas the concentrations of CORT showed a reverse pattern. The frequency and duration of aggression (indicative of social status) increased with elevated T concentrations. Sperm quality in terms of number and activity were associated with higher concentrations of serum T in dominant gerbils, whereas small sperm counts and poor-quality sperm were associated with relatively higher concentrations of serum CORT in subordinate gerbils. Together, our data indicated that there was no trade-off between pre- and post-copulatory sexually selected traits but hormones orchestrated the relationship between these traits in male Mongolian gerbils.

1. Introduction

Darwin's theory of sexual selection suggests that male mating competition (pre-copulatory competition) is a potent selective pressure for the evolution of secondary sexual traits such as ornaments, weapons or chemical signals. However, when females mate with more than one male, this male–male competition extends to post-copulation which affects paternity through sperm competition [1]. Sperm competition can also be an important selective pressure for the evolution of ejaculate quality and genital morphology [2]. Consequently, a male's overall fitness was affected by pre- and post-copulatory competitions, and the relationship has crucial implications for the evolution of male reproductive strategies.

Males can allocate only a limited amount of resources to reproduction as almost all life-history traits depend upon the same resources [3]. The expression and maintenance of sexual traits involved in both pre- and post-copulatory competitions are both energy and time consuming [4,5]. Therefore, Parker's sperm competition model predicted

* Corresponding author at: Institute of Zoology, Chinese Academy of Sciences, 1 Beichen Xilu, Chaoyang, Beijing 100101, China.
E-mail address: zhangxy@ioz.ac.cn (X.-Y. Zhang).

http://dx.doi.org/10.1016/j.physbeh.2015.02.041
0031-9384/

that investment into elaboration in ejaculate quality (such as testes, sperm quantity and quality) could reduce the availability of resources for all other reproductive traits (such as social status and scent-marking behavior) [3]. Given this, we might predict that subordinate males could counteract an initial disadvantage in pre-copulatory competition by adopting less-obvious post-copulatory tactics that enhance sperm competitive success [1,3]. However, empirical studies have revealed considerable inconsistency in the direction and strength of relationship among these sexual traits [6,7]. Because energy is a crucial resource for all life-history traits, variation in the rate of energy acquisition and transformation (the metabolic rate) is an important issue within the framework of evolution and ecological physiology [8,9]. Moreover, few studies have illustrated the costs of social status and sexual traits from the aspect of energetics.

Sex steroids (testosterone, T) and stress hormones (glucocorticoids, GCs; corticosterone, CORT, in rodents) are ubiquitous among male vertebrates and profoundly affect both reproductive behavior and reproductive physiology [10,11]. Circulating concentrations of T are elevated during the breeding season (especially during the courtship and mating phase) in many vertebrate species [12]. Gonadal T controls seasonal changes in reproductive processes such as sexual (vocalization, courtship, copulation) and aggressive (mate-guarding, territorial) behaviors, as well as the expression of numerous sexually selected traits and sperm production and maturation [12,13]. GCs suppress reproductive functions along the hypothalamic–pituitary–gonadal (HPG) axis at multiple levels: 1) the testis/ovary (to modulate steroidogenesis and/or gametogenesis directly); 2) the pituitary gland (to inhibit the release and synthesis of follicle-stimulating hormone and luteinizing hormone); and 3) the hypothalamus (to decrease the release and synthesis of gonadotropin-releasing hormone) [14]. To our knowledge, no study examined how hormones orchestrate the relationship between pre- and post-copulatory sexually selected traits.

Mongolian gerbils (*Meriones unguiculatus*) are distributed throughout the agricultural land and desert grassland of Mongolia, Northern China and Russia [15]. They are small non-hibernating, seasonally breeding, and granivorous rodents which live in burrow systems or social groups consisting of multiple females and males year-round [16,17]. In the field the scent marking behavior of Mongolian gerbils was done by rubbing mid-ventral sebaceous glands against substrates within their territory and was found to be modulated by T [18,19]. Male gerbils, especially the largest dominant males, mark their territory to attack intruder males and to attract females in estrus [20]. During the breeding period Mongolian gerbils overlapped more home ranges which allows male gerbils to enter neighboring burrow systems to mate with extra-pair females [21] and female gerbils to access male mates of neighboring colonies [20], suggesting that they possessed a characteristic of promiscuous species. In the present study, we examined the individual difference in energy intake, resting metabolic rate (RMR), gland mass, sperm quality and quantity, serum CORT and serum T concentrations to test the hypothesis that there was a trade-off between pre- and post-copulatory sexually selected traits, and CORT and T orchestrate the relationship of these traits in male Mongolian gerbils.

2. Materials and methods

2.1. Animals

The animals were the offspring of Mongolian gerbils trapped in 1999 in Inner Mongolian grasslands. They were housed in plastic cages (3 or 4 male gerbils/cage, 30 × 15 × 20 cm) with sawdust as bedding prior to experiment, and maintained under a constant photoperiod of 16 h:8 h light–dark cycle and at a temperature of 23 ± 1 °C. They were provided with commercial standard rat pellets (Beijing HFK Bioscience Co.) and water ad libitum. Sixteen adult male gerbils (weight-matched) were selected from different cages. Energy intake and RMR were measured in these gerbils. They were paired randomly (never met before), and then their dominant–subordinate relationship was established under chronic social encounters in a neutral arena. Finally, serum hormone concentrations and sperm quality and quantity were evaluated. All experimental procedures complied with the guidelines of the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences.

2.2. Energy intake

Food intake was measured in metabolism cages as previously described [22]. Briefly, food was quantitatively provided then feces and food residues were collected at same time in the fourth day, and then dried to constant mass at 60 °C. The difference between the food given and residue was defined as dry matter intake (DMI). A Parr 1281 oxygen bomb calorimeter (Parr Instrument, USA) was used to determine the caloric value of food and feces. The following equations were used to calculate gross energy intake (GEI, kJ/g), digestible energy intake (DEI, kJ/g) and digestibility of energy [22,23]:

$$\text{GEI} = \text{DMI} \times \text{caloric value of food};$$
$$\text{DEI} = \text{GEI}–(\text{dry mass of feces} \times \text{caloric value of feces});$$
$$\text{Digestibility}(\%) = \text{DEI}/\text{GEI} \times 100\%.$$

2.3. RMR

RMR was measured using an open-flow respiratory system (TSE LabMaster Calorimetry System, Germany), following previous studies [24]. Briefly individual gerbils were transferred into a respiratory chamber (TSE, type I for mice, volume 2.7 L) housed inside a constant-temperature incubator (30 ± 0.5 °C, Sanyo, MIR-554). Fresh air was warmed by a copper tube to 30 °C and then pumped through the animal chamber at a flow rate of 800 mL/min. Subsequently, air leaving the chamber passed through a gas analyzer at the rate of 390 mL/min. Each measurement lasted 3 h with data recorded every 6 min. RMR was quantified as the oxygen consumption (VO_2; mL/min) calculated as the average of 12-min stable lowest values. Body mass was recorded before the measurement.

2.4. Behavioral procedures

Sixteen adult males were selected and assigned into 8 fixed pairs with similar body weights (within 10% difference). The dominant–subordinate relationship was formed very quickly in a neutral arena (40 × 27.5 × 60 cm Plexiglas box). The arena was divided into equal compartments using a removable opaque partition following the previous protocols [25]. Briefly, each gerbil was acclimated for 5-min period in each compartment before the partition was removed, and then they were allowed to interact freely for 10 min during which their encounters were recorded using a digital video. The arena was thoroughly cleaned with water and 75% ethanol before or between trials. Frequency and duration of aggression (chasing, sideways posture, attack, biting and latency of the initial attack), defense (cowering, upright posture, threatening, fleeing, and lying on the back on the ground), sniffing, self-grooming, locomotion displayed by each animal were quantified, as defined previously [25–27]. Behaviors were continuously recorded by hand on a data sheet with a pre-calibrated time scale in units of 10 s. Behaviors that lasted 10 s or less was treated as 1 unit, if the duration was between 10 s and 20 s which was considered to be 2 units, and so on [26]. Behavioral procedures were repeated for 7 consecutive days once each day for each pair.

2.5. Blood sample and organ mass

Following behavioral procedures, animals were sacrificed by CO_2 overdose. Blood samples were collected and then centrifuged at 4 °C