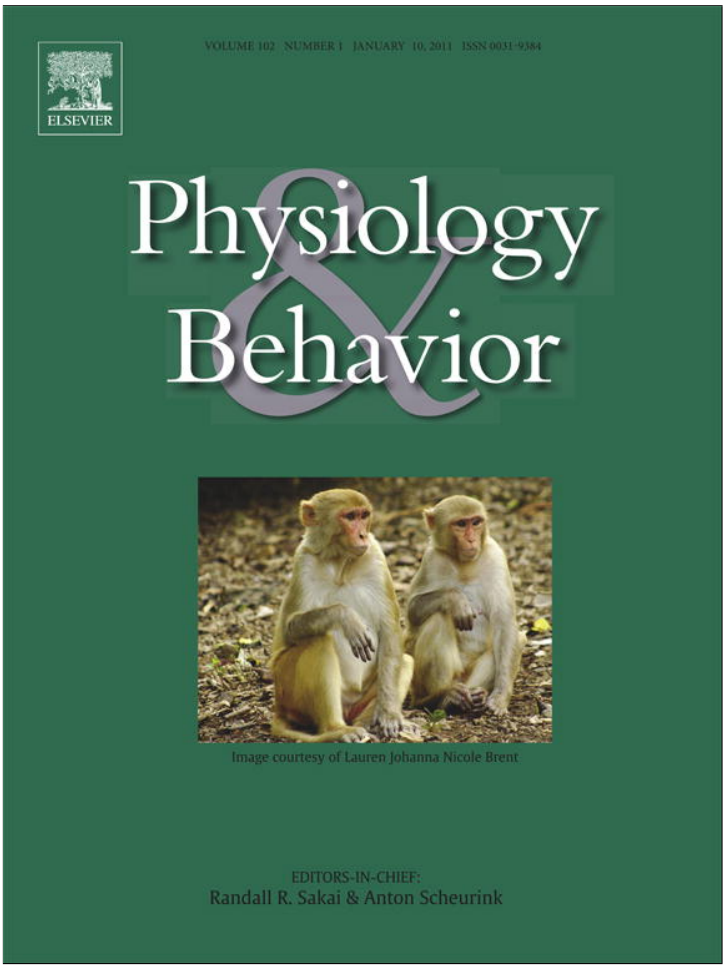

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"Nice guys finish last": Influence of mate choice on reproductive success in Long–Evans rats

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ABSTRACT

The present study was designed to determine if male physiology and male reproductive behavior predict reproductive success in Long–Evans rats. Mating behavior was observed in sexually naïve, naturally cycling female rats during behavioral estrous that were given the opportunity to mate with two males simultaneously. DNA analysis of offspring born following these mating encounters was used to identify the paternity of each pup. In order to assess the effect of mate choice during these mating encounters on reproductive success, one male rat in each pair was categorized as the preferred mate if the female spent more time (>50%) with him during the mating test of the present study. Furthermore, each male in the pairs was categorized as "attractive" or "non-attractive" by computing the number of females that preferred each male across many mating tests. Similar to results reported in Lovell et al. (2007), during 76% of these mating tests the same male rat in each pair was preferred by different female rats. Overall attractiveness of individual male rats predicted reproductive success in the present study. Interestingly, "attractive" males sired significantly FEWER pups than "non-attractive" males. Neither behavioral (e.g., latency to first sexual stimulation, number of sexual stimulations) nor physiological measures (e.g., body weight, urinary testosterone levels) of male rats predicted their reproductive success. In conclusion, the present results indicate that certain features of some males are more attractive to females, but attractive males are at a reproductive disadvantage (as measured by the number of pups sired). Although basal urinary testosterone levels did not differ between males that sired the majority of pups in a litter and males that sired few or none of the pups in a litter, aggression and/or other physiological measures of fertility (e.g., penile reflexes) may differ between males that are attractive to females and those that have a reproductive advantage.

1. Introduction

Rats are a polygynandrous species, such that both males and females mate with multiple partners simultaneously [1–3]. In naturalistic and semi-naturalistic conditions, female rats are able to control the timing and number of sexual stimulations received by approaching and withdrawing from their male partners, a phenomenon known as paced mating behavior [4]. When female rats pace the receipt of sexual stimulation, fewer intromissions are required to achieve the progestational state necessary for pregnancy and they are subsequently more likely to become pregnant than females that cannot control the receipt of sexual stimulation [5]. Therefore, paced mating behavior is advantageous for reproductive success.

When female rats are given the opportunity to pace the receipt of sexual stimulation from two males simultaneously, they display a preference for one male over the other by spending more time with one male and returning to him more quickly after receiving sexual stimulations [6]. A female rat's preference for a particular male is consistent across repeated encounters with the same pair of males (i.e., preferring the same male 70% of the time). In addition, the preference of different females for one male in a particular pair is consistent; that is, approximately 70% of all females prefer the same male in any given pair. Consequently, Lovell et al. [6] concluded that some males may possess traits (e.g., physiological, genetic, behavioral) that make them more attractive than other males to the majority of females.

Similar to pacing the receipt of sexual stimulation, female mate choice may have adaptive significance by increasing reproductive success when certain pairs of animals mate. In fact, Drickamer and colleagues [7] demonstrated that female mice mated with preferred mates produced more litters than female mice mated with non-preferred mates. Furthermore, offspring born from mating encounters of preferred mates were more dominant and built better nests than offspring sired by non-preferred mates. Likewise, female mice prefer males with genetic resistance to infection over males that are genetically susceptible to infection [8]. Thus, in some situations, mate choice may affect reproductive success and offspring health.

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Mate choice is a form of pre-copulatory sexual selection [9], wherein sexually selected male traits may act as indicators of genetic quality [7]. Dominance and secondary sexual characteristics may advertise a male's reproductive fitness to females; however, relationships between these traits and reproductive success are often contradictory between different species and across various studies. For example, secondary sexual characteristics in promiscuous Soay sheep serve as good predictors of reproductive success. Specifically, males with larger horns and testes sire more offspring [10]. On the other hand, Bangham and colleagues [11] found no relationship between testes size and reproductive success in *Drosophila melanogaster*. In the yellow-toothed cavy (*Galea musteloides*), a promiscuous South-American rodent, dominant males sire significantly more offspring than subordinate males [12]. However, social status did not predict reproductive success in Norway rats, as dominant and subordinate males sired similar proportions of pups when allowed free access to a female (who was housed in their home cage) [13]. Therefore, subordinate male rats are able to reproduce at the same rate as dominant male rats despite their social status.

Because rat litters can have multiple paternities [14], competition for impregnating a female does not necessarily stop at copulation. Post-copulatory sexual selection involves competition between the sperm of multiple males inside of the female's genital tract. Promiscuous mating systems and the resultant sperm competition are advantageous for females. For example, female yellow-toothed cavies that mated simultaneously with multiple males had lower rates of offspring mortality than females that mated with only one male [12]. Sperm competition may select for certain male traits and behaviors that increase reproductive success, such as large testes, high sperm motility, high concentration of sperm per ejaculation, strong penile reflexes or fast rates of copulatory behavior [15].

Because multiple intromissions are necessary to initiate the progestational state in female rats, male rats must be able to perform intromissions as quickly as possible to have the best chance of impregnating a female. When mating in the presence of competitor males (i.e., when sperm competition is high), non-preferred male rats (i.e., males with whom females spend less time) have been shown to require fewer intromissions to reach an ejaculation as well as less time to achieve intromissions and ejaculations [16]. However, similar to subordinate male rats [13], non-preferred mates are able to reproduce at rates similar to preferred mates [17].

When sperm competition occurs, males at a disadvantage (e.g., subordinate, non-preferred) may employ a number of strategies to overcome obstacles to reproductive success. One mechanism, sperm allocation (i.e., altering total number of sperm per ejaculate), may have been selected for because the proportion of offspring sired increases with the number of sperm ejaculated relative to competitors [18]. Both male meadow voles [19] and male Norway rats [20] respond to the risk of sperm competition by increasing sperm allocation. Interestingly, sperm allocation is sensitive to the quality of the male competitor, with less sperm allocated if the competitor is less of a threat, such as when the competitor is food-deprived [21].

Sexual traits, such as those selected for by sperm competition, are often related to testosterone levels. For example, penile reflexes are restored in castrated male rats treated with testosterone [22]. Intromission and ejaculation latencies are shorter when intact male rats are treated with testosterone [22]. In addition, testosterone treatment in senescent rats stimulates spermatogenesis [23]. Because testes produce both testosterone and sperm, it is not surprising that testes size has been shown to be positively correlated with testosterone levels, as well as spermatogenesis [24,25]. Garamszegi and colleagues [24] proposed that larger testes may have evolved primarily to produce more sperm and thus, increase the likelihood of fertilization. Moreover, increased testosterone may have evolved secondarily to allow males to defend their mates during aggressive encounters.

Recently our lab has investigated the relationship between female mate choice and male reproductive success in Long–Evans rats [17]. Although mate choice reflects an aspect of female sexual motivation, female preference did not predict which mates would sire more pups in a litter. Therefore, the current study was designed to further investigate additional factors that may contribute to the differential reproductive success of some males in pairs of cohabitating rats that are competing during a sexual encounter. Physiological and behavioral measures of the male rats were assessed to determine the relationships between basal urinary testosterone levels, testes size, male copulatory behavior and success siring pups. Furthermore, because males in the present study were mated with many additional female rats in other experiments, the relationship between an overall measure of "attractiveness" (similar to the measure observed in Lovell et al., [6]) and success siring pups was also investigated.

2. Method

2.1. Subjects

Sixteen sexually naïve female Long–Evans rats (200–300 g) and 32 sexually experienced male Long–Evans rats (400–600 g) were used as subjects. Rats were purchased from Harlan Sprague–Dawley (Indianapolis, IN) and were housed in hanging plastic cages with aspen wood shavings for bedding and food and water available ad libitum. The male rats were profiled genetically using DNA microsatellite markers, which have been validated by Harlan GenScreen (Indianapolis, IN). The male rats were pair housed upon arrival such that the males of each pair had distinct genetic profiles based on microsatellite analysis, which facilitated paternity identification of offspring. Although all males were born on the same day, each male rat was taken from a different litter; and therefore, were not siblings. The female rats, also purchased from Harlan Sprague–Dawley, were not related to the male rats. The female rats were housed three to a cage. All rats were weighed weekly. Temperature and humidity in the rat colony room were monitored, and the lights were maintained on a reversed 12:12 h light–dark cycle (lights off at 10:00 a.m.). All experiments were conducted during the dark cycle under dim red light.

2.2. Estrous cyclicity

The female rats were monitored for one month using vaginal cytology to ensure normal estrous cyclicity. Vaginal cytology was examined once daily at 8:00 a.m. by collecting vaginal secretions using a sterile plastic pipette filled with saline [26]. Vaginal fluid was placed onto glass slides and examined under a microscope. Female rats were recorded as being in proestrous, estrous, metestrous, or diestrous based on the proportion of cell types. Proestrous vaginal secretions consisted mainly of nucleated epithelial cells; estrous secretions consisted of cornified nonnucleated cells; metestrous secretions consisted of equal proportions of round leukocytes, cornified, and nucleated epithelial cells; and diestrous secretions consisted mostly of round leukocytes [26]. After one month of monitoring, the female rats were mated in the afternoon (~1:00 p.m.) with a pair of cohabitating male rats if their morning vaginal secretions determined that they were in proestrous [27]. On occasion, female rats were not receptive during mating tests even though vaginal secretions observed in the morning indicated that they would be in behavioral estrous in the afternoon. When this happened, the female rat was returned to her home cage and estrous cyclicity was monitored daily until she was in proestrous again. Furthermore, if a female rat was determined not to be pregnant three weeks after the mating test, she was mated again with the same pair of male rats until she became pregnant (up to three repetitions).

2.3. Urine collection

Each male rat was acclimated to the chambers used to collect urine for a minimum of three sessions (two hours each) before any samples were collected for analysis. Each rectangular chamber was made of one Plexiglas front wall that opened, three aluminum sidewalls, an aluminum ceiling (25.5 cm long × 24 cm high 30.5 cm wide) and a metal grid floor (1.3 cm between bars), which had a removable Plexiglas tray underneath. The chambers were cleaned after each session with a 50% ethanol solution. All urine samples were collected during a 4-week period. Approximately one week after mating tests were conducted, each male rat was placed in the chamber for two hours until urine samples were observed in the tray underneath the floor. If no urine was observed during the 2-hour session, the males were returned to their home cage and observed again until samples could be collected (every 24 h). Samples were collected from most males after one or two sessions. The samples were collected using a plastic pipette and kept on dry ice until they could be stored at −80 °C.

2.4. Testosterone assay

Urinary testosterone was measured by treating an aliquot of urine with 10,000 U of β-glucuronidase (*H. pomatia*) overnight and assayed using an enzyme immunoassay (EIA) kit (Oxford Biomedical Research, Inc.). The cross-reactivity of the antibody is as follows: testosterone 100.0%, dihydrotestosterone 100.0%, androstenedione 0.86%, testosterone enanthate 0.13%. The percentage for other steroid metabolites is less than 0.12%. Creatinine concentrations were measured to compensate for differences in urine production (Oxford Biomedical Research, Inc.). Urinary testosterone values are expressed as the amount of testosterone in nanograms per milligram of creatinine.

2.5. DNA extraction and paternity identification

Pinna snips were collected from all pups (25–60 days old) and mother rats. A piece of pinna approximately 3 mm long and 1 mm wide was cut off one ear of each rat with a clean, sharp scissor. No anesthesia was required for the procedure. Genomic DNA was extracted from the tissue samples for microsatellite DNA analysis (Harlan GenScreen). Paternity identification of the offspring was determined as previously described [17]. Briefly, each potential father was determined by comparing the electropherogram pattern of the offspring to those of the two possible fathers and the mother.

2.6. Testes dissection

After mating tests were completed and urine was collected, the male rats were euthanized with an i.p. injection of sodium pentobarbital (150 mg/kg). Both testes were then dissected from the scrotum of each male rat, and the testes were weighed without the epididymis. To correct for body weight differences, a testes-to-body-weight ratio was also calculated for each male rat (i.e., wet testes weight divided by body weight).

2.7. Behavioral procedure

2.7.1. Acclimation

All rats were acclimated to the mating chambers on two separate occasions for 15 min each prior to mating tests. Each mating chamber consisted of a Plexiglas arena (101.0 cm long × 32.0 cm high × 37.0 cm wide) divided into three equal compartments using clear Plexiglas dividers. Each of the dividers had a 5.0 cm hole in each of the two bottom corners. Wood shavings covered the floor of each compartment. During acclimation sessions for the male rats, a single male rat was placed in each of the outer compartments and was tapped lightly on the nose if he attempted to exit through the holes in the dividers. Because of this training and their size, male rats did not leave their compartments during mating tests. During acclimation sessions for the female rats, a single female rat was placed alone in the chamber and allowed to move freely between the three compartments.

2.7.2. Mate choice

On the afternoon of proestrous, female rats were given the opportunity to mate with two male rats simultaneously. Prior to the start of each mating test, a female rat was placed in the center compartment of the mating chamber. Solid opaque dividers prevented the female from entering either of the two side compartments. Two male rats (that were pair housed) were placed randomly into either of the side compartments. All rats were acclimated to the mating chamber for 5 min.

The mating test began when the opaque dividers were removed, thereby providing the female rat access to both male rats simultaneously. The mating test was ended when the female rat received an ejaculation from each male and then returned at least once to visit each male. At this point, the opaque dividers were replaced and the rats were returned to their home cages. It was possible for one male rat to ejaculate multiple times before a test was ended. For example, a female rat could receive multiple ejaculations from one male before she received an ejaculation from the other male. On occasion, a male would fail to ejaculate within 60 min of the test starting. This was likely due to the female spending only brief amounts of time with that male. If after 60 min a male rat had not ejaculated, the opaque dividers were replaced, the timer stopped and the female rat was confined to the male rat's compartment until she received an ejaculation. This procedure was used to allow sperm competition between the male rats. Although sperm competition could not be equated, it was still possible for any male who failed to ejaculate within the first 60 min to ejaculate and still sire pups if he was given this additional time confined with the female.

Trained observers, who sat approximately 1.0 m in front of the arenas, used a stopwatch to record the type and timing of sexual stimulations (i.e., mounts, intromissions, ejaculations), solicitation behaviors (i.e., hops and ear wiggles), rejection behaviors (i.e., kicks, squeaks, and defensive postures), and entries into and exits from each compartment. Compartment entries were scored when all four paws of the female rat passed through the holes in the clear Plexiglas dividers. Time spent in each compartment and percentages of time spent with each of the male rats were calculated, and the male rat that the female rat spent the greatest amount of time with was classified as the preferred mate. The lordosis response (LR) of the female rat to each sexual stimulation was scored on a 4-point scale (0–3) and the lordosis quotient (LQ) was calculated as the percentage of lordosis responses of 2 or 3 [28]. In addition, contact-return latency and percentage of exits in response to each type of sexual stimulation received from each male rat were calculated. Contact-return latency represents the time elapsed between receiving sexual stimulation, leaving the male rat's compartment, and re-entering the male rat's compartment. If multiple sexual stimulations were received during a visit to a male rat, contact-return latency could only be calculated for the last stimulation received before the female rat left the male rat's compartment. It is important to note that time spent alone and time spent with the other male is included in this measure of latency. Percentage of exits represents the likelihood that the female rat left the male rat's compartment following the receipt of sexual stimulation.

A number of male mating measures were calculated in order to assess characteristics of the male rats that may contribute to differential reproductive success. The total number of sexual stimulations (i.e., mounts, intromissions, and ejaculations) performed by each male rat was summed as well as divided by time the female spent with the male to calculate a rate of stimulations. Latency to achieve the

first sexual stimulations (e.g., first mount, first intromission, and first ejaculation) after the start of the mating test was recorded for each male rat. Hit rate [(# of intromissions + # of ejaculations)/(# of mounts + # of intromissions + # of ejaculations) × 100] and the average interval between intromissions (inter-intermission-interval; III) in seconds were calculated. A male rat was classified as having a reproductive advantage if he sired more pups than the other male rat in the pair (a male was considered to have a reproductive advantage even if he sired only one more pup than the other). Finally, an attractiveness ratio [6] for each male was calculated based on all of the mating tests each male participated in before, after and including the mating test of the present study (# of tests male was preferred/total number of mate choice tests male participated in × 100). Any male with an attractiveness ratio greater than 51 was categorized as "attractive". Any male with an attractiveness ratio less than 49 were categorized as "non-attractive". All mating tests were recorded with digital video cameras (Sony DCR-HC65) for posttest analysis of behaviors.

2.8. Parturition

Following each successful mating test, female rats were given nesting material and monitored daily for signs of pregnancy and parturition. Around the time of parturition, we observed mothers every 8 h to find any evidence of cannibalism. All females who became pregnant (n = 15) gave birth approximately 22 days after mating, at which time pups were counted. Pups were weaned at 21 days old, at which time male and female offspring were placed into separate cages.

2.9. Data analysis

All measures of female mating behavior were analyzed by comparing behaviors with the preferred mate to behaviors with the non-preferred mate using paired *t*-tests. The preferred mate was defined as the male with whom the female spent more time with during the mating test (>50% of the test duration). To analyze the magnitude of this preference, preference ratios were calculated as $T_P/(T_P+T_N)$, where T_P = time with preferred mate and T_N = time with non-preferred mate. This ratio is equal to $50\% + 0.5\ (T_P - T_N)\ /\ (T_P + T_N)$. If T_P and T_N were only the result of chance times from a normal distribution with mean μ_T and variance σ_T^2, then the value $0.50\ (T_P - T_N)$ would have a half-normal distribution with an expected value of $\sigma_T/\sqrt{\pi}$ and the value $(T_P + T_N)$ would have an expected value of $2\mu_T$. Thus, the expected preference ratio that would have resulted from chance was calculated as $0.50 + \sigma_T/2\mu_T\sqrt{\pi}$ and was compared to the observed preference ratio using a one-sample *t*-test.

Independent *t*-tests were used to assess differences between male rats in four categories (sired more pups vs. sired fewer pups; ejaculated first vs. ejaculated last; preferred vs. non-preferred; attractive vs. non-attractive) on physiological measures (number of pups, urinary testosterone, testes weight, body weight and testes-to-body-weight ratio) and/or male behaviors recorded (e.g., rate and number of stimulations, latency to achieve first stimulation, hit rate, III). Correlation coefficients were calculated on physiological measures (e.g., testes weight, testes-to-body-weight ratio, urinary testosterone, number of pups sired).

3. Results

3.1. Paternity

Of the 16 female rats that were mated with two males simultaneously, 15 were successfully impregnated and gave birth to pups. One female rat became pregnant but consumed her pups (n = 5). Cannibalism of approximately 3% of the total number of pups born is comparable to percentages reported in other studies [29,30]. A total of 149 pups were born and survived to weaning. Paternity was identified in all 149 pups. In most of the families, the preponderance of pups was sired by just one of the male rats (Table 1). Two males failed to ejaculate within 60 min of the start of the mate choice test. However, both males ejaculated in less than a minute when the female was confined to their compartment, and therefore each was given an ejaculation latency of 3600 s.

3.2. Reproductive Advantage, Male Mating Behavior and Reproductive Physiology

There were no statistically significant differences between male rats who had a reproductive advantage (i.e., sired significantly more pups) and those that did not (7.9 pups ± 1.0 vs. 2.6 pups ± 0.8, respectively) on any male copulatory behaviors measured (DATA NOT SHOWN). Although there was a significant correlation between the number of pups sired and the male rat's testes weight, $r(28) = 0.41$, $p < 0.05$, the correlation between the number of pups sired and the male rat's testes-to-body-weight ratio was not significant, $r(28) = 0.32$, $p = 0.095$ (Table 1). Previous research suggested that testes weight would correlate positively with testosterone levels [24,25,31], however no significant relationship between urinary testosterone (i.e., testosterone-to-creatinine ratios) and testes weight (wet weight or corrected for body weight) was observed in the present study (wet weight: $r(28) = -0.29$, $p = 0.13$.; testes-to-body-weight ratio: $r(28) = -0.23$, $p = 0.25$)

Furthermore, the male rats that ejaculated last did not have a reproductive advantage over the male rats that ejaculated first. Specifically, on average, male rats that ejaculated last sired 4.5 ± 1.2 pups, whereas male rats that ejaculated first sired 5.4 ± 1.0 pups. However, male rats that ejaculated first had more ejaculations on average (1.9 ± 0.3) than male rats that ejaculated last (1.00 ± 0.00) $t(28) = 2.82$, $p < 0.05$. If multiple ejaculations were achieved by the male rat that ejaculated first, they all occurred before the other male rat was able to ejaculate once. No other measures of male mating behaviors differed between males with a reproductive advantage and those without (all *t*'s < 2.0).

3.3. Mate Choice and Female Mating Behavior

As expected, female rats spent significantly more time with one male (their preferred mate) than another (their non-preferred mate), (Fig. 1*top*) $t(14) = 4.22$, $p < 0.001$. It is worth noting that the percentage of time spent with male stimulus animals is comparable to what has been observed previously in female rats tested for mate choice [6,17]. Based on the time spent with either male rat, the mean preference ratio for the female rats was 0.76 ± 0.05, which is significantly greater than chance (0.59; $t(27) = 3.64$, $p < 0.05$). Female rats visited their preferred mate significantly more than their non-preferred mate, (Fig. 1*middle*) $t(14) = 4.72$, $p < .01$, and displayed significantly more solicitation behaviors towards their preferred mate in his compartment than towards their non-preferred mate, (Fig. 1*bottom*) $t(14) = 2.83$, $p < .05$. Female rats were less likely to leave their preferred mate after receiving a mount (Preferred: 69.0 ± 9.2% vs. NON-Preferred: 89.6 ± 4.8%), $t(9) = 2.5$, $p < 0.05$. Although in previous studies we have found consistently that a female rat returns to her preferred mate faster after leaving his compartment than she returns to her non-preferred mate [6,17], no significant differences in contact-return latencies after mounts (Preferred: 20.3 ± 4.3 s vs. NON-Preferred: 73.5 ± 23.5 s; $t(9) = 2.2$, $p > 0.05$) or after intromissions (Preferred: 39.3 ± 11.3 s vs. NON-Preferred: 103.9 ± 53.1 s; $t(12) = 1.2$, $p > 0.05$) were observed in the present study when we used a two-tailed test. However, because we predicted *a priori* that there would be differences based on our previous findings [6,17], we used a one-tailed test of significance and found

Table 1
Behaviors observed during mate choice tests and male physiological measures for each individual family.

	# of pups	# of ejacs	Preference	Attractiveness	First to ejac	Ejac latency (s)	Testes weight (g)	Testes-to-body-weight ratio	Testosterone to creatinine ratio (ng/mg)
Family 1									
Male #1	5	4	Preferred	Attractive	First	269	3.66	.71	1.21
Male #2	7	1	–	–	–	2613	3.41	.67	1.40
Family 2									
Male #1	11	1	Preferred	Attractive	First	229	N/A*	N/A*	1.60
Male #2	0	1	–	–	–	546			1.51
Family 3									
Male #1	5	1	–	N/A**	–	451	3.63	.65	1.77
Male #2	4	1	Preferred		First	190	3.28	.58	3.83
Family 4									
Male #1	4	3	Preferred	Attractive	First	226	3.73	.62	1.57
Male #2	9	1	–	–	–	2739	3.24	.57	1.36
Family 5									
Male #1	1	1	Preferred	Attractive	–	1246	3.49	.64	1.17
Male #2	6	1	–	–	First	988	3.42	.57	1.58
Family 6									
Male #1	0	1	Preferred	Attractive	First	1542	3.30	.60	2.84
Male #2	11	1	–	–	–	2792	4.05	.67	1.60
Family 7									
Male #1	0	2	Preferred	Attractive	First	290	3.53	.54	1.26
Male #2	10	1	–	–	–	1419	3.56	.72	1.80
Family 8									
Male #1	12	4	Preferred	–	First	111	3.84	.69	1.52
Male #2	0	1	–	Attractive	–	3600	3.02	.50	2.37
Family 9									
Male #1	8	1	Preferred	–	First	383	2.94	.61	1.80
Male #2	0	1	–	Attractive	–	845	3.58	.60	2.00
Family 10									
Male #1	2	1	Preferred	Attractive	First	292	3.62	.64	2.36
Male #2	11	1	–	–	–	1237	3.81	.60	2.02
Family 11									
Male #1	12	1	–	–	First	350	3.84	.63	1.23
Male #2	0	1	Preferred	Attractive	–	808	3.68	.64	1.50
Family 12									
Male #1	8	4	Preferred	Attractive	First	379	3.62	.60	1.15
Male #2	3	1	–	–	–	3600	3.32	.51	2.09
Family 13									
Male #1	4	3	Preferred	Attractive	First	243	3.88	.69	1.78
Male #2	9	1	–	–	–	1620	3.76	.55	0.92
Family 14									
Male #1	0	1	Preferred	Attractive	–	501	2.95	.48	1.33
Male #2	3	1	–	–	First	456	3.46	.55	2.86
Family 15									
Male #1	2	1	–	Attractive	–	1164	3.50	.59	0.78
Male #2	2	1	Preferred	–	First	835	3.72	.74	0.90

*Unable to obtain testes weight
**Males were equal in attractiveness

that females returned to their preferred mates significantly faster than to their non-preferred mates after mounts ($p<0.05$).

No other measures of paced mating behavior differed between behaviors directed towards preferred and non-preferred mates (all t's<1.9).

3.4. Mate Choice, Male Mating Behavior, and Reproductive Success

Preferred mates had shorter III than non-preferred mates overall, $t(23)=2.34$, $p<0.05$. Preferred mates achieved more intromissions, $t(28)=3.23$, $p<0.05$, and achieved their first intromission faster than non-preferred mates, $t(26)=2.15$, $p<0.01$ (Table 2). Preferred mates also achieved more ejaculations, $t(28)=2.96$, $p<0.05$, and achieved their first ejaculations faster than non-preferred mates, $t(28)=3.57$, $p<0.01$ (Table 2). However, preferred mates also had more access to the females because each female rat spent a greater proportion of the mating test with their preferred mate ($26.0\pm4.5\%$) than with their non-preferred mate ($6.3\pm1.1\%$), $t(28)=4.27$, $p<0.01$. Furthermore, when rates of sexual stimulations were calculated based on how much time each male had access to the female

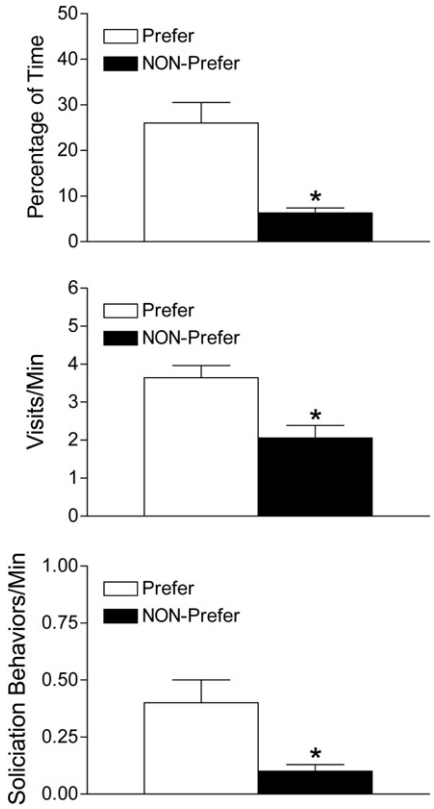

Fig. 1. *Top*: Female rats spent significantly more time with their preferred mate than their non-preferred mate during the mating test. *Middle*: Female rats made significantly more visits to their preferred mate than their non-preferred mate. *Bottom*: Female rats displayed significantly more solicitation behavior when in the vicinity of their preferred mate than their non-preferred mate. Means are reported ± standard error of the mean for the 15 females that successfully reproduced. An asterisk indicates a significant difference between preferred mates and non-preferred mates ($p<.05$ paired *t*-tests).

a very different pattern of results are observed. Non-preferred mates display a higher rate of stimulations. For example, preferred mates displayed 0.007 ± 0.001 ejaculations/s, whereas non-preferred mates displayed 0.02 ± 0.003 ejaculations/s, $t(28) = 4.07$, $p<0.01$, suggesting that males at a disadvantage are more efficient. Rate of mounts and intromissions were also faster for non-preferred mates (mounts: 0.04 ± 0.01; intromissions: 0.06 ± 0.01) than preferred mates (mounts: 0.02 ± 0.004; intromissions: 0.03 ± 0.007); however, these differences did not reach statistical significance (rate of mounts: $t(28) = 2.01$, $p = 0.055$; rate of intromissions: $t(28) = 2.00$, $p = 0.055$). Despite these differences in performance, neither preferred mates nor non-preferred mates displayed a significant reproductive advantage (Table 2). In fact, only 5 of the 15 preferred mates sired more pups than non-preferred mates. No other measures of male mating behavior differed between preferred and non-preferred mates (all *t*'s<2.0).

3.5. Attractiveness, Male Mating Behavior and Reproductive Success

Physical, behavioral or genetic traits that distinguish two males from one another may reflect differential reproductive success. Because the 15 pairs of male rats used in the current study were tested in additional mate choice tests with many different hormonally-primed, ovariectomized females in other experiments (during the same time frame that the present study was conducted), we were able to determine if attractiveness of a particular male represents one such trait. Therefore, we examined patterns of female preferences for a particular male in any given pair. The proportion of tests in which one male from the 15 pairs was classified as preferred was calculated. Each pair of males was tested for a minimum of 4 mating tests with a different female in each test (average number of tests: 5.0 ± 0.3; Range 4–7). Based on these mating tests, an attractiveness ratio for each male was calculated [6]. Both male rats in one pair were preferred the same number of times; therefore, they were excluded from this data analysis. In the remaining 14 pairs of males, one male in each pair was classified as the preferred mate by more females than the other male and classified as "attractive", a result that has been previously reported [6]. All of the attractive males had an attractiveness ratio greater than 51 (Mean ± SEM: 76.1 ± 6.8). In other words, attractive males were preferred by 76.1% ± 6.8 of the females, whereas non-attractive males were preferred by 23.7% ± 6.8 of the females. Using the categorization of "attractive" and "non-attractive" compiled from these tests, physiological measures (e.g., testosterone, testes-to-body-weight ratio) and reproductive success were analyzed with independent *t*-tests.

Only one difference reached statistical significance; that is non-attractive males sired significantly more pups than attractive males (7.0 ± 1.1 pups vs. 3.3 ± 0.9 pups), $t(26) = 2.41$, $p<.05$. No physiological measures (e.g., testes weight, testosterone) differed between attractive and non-attractive males (all *t*'s<1.1).

4. Discussion

In the present study, female rats spent more time with one male than another when given the opportunity to mate with two males simultaneously, visiting their preferred mate more frequently than their non-preferred mate. These results are consistent with previous findings [6,17,32]. The female rats also displayed more solicitation behaviors towards their preferred mate than their non-preferred mate. This is the first study to report that female rats display more solicitation behaviors when they are in the vicinity (i.e., compartment) of their preferred mate. Others have suggested that the rate of solicitation behaviors is one of the many indications of female sexual motivation in rats [4,33]. The results of the current study further support this conclusion. The female rats in the current study did not return to their preferred mate significantly faster than to their non-preferred mate following intromissions. However, the pattern of behavior was similar to what was observed previously [6,17]. Because female rats displayed evidence of enhanced sexual motivation directed towards the preferred mate (i.e., more solicitation behaviors, more visits), it is likely that the lack of significant results for contact-return latency

Table 2
Behaviors observed during mate choice tests and male physiological measures. Means are reported ± standard error of the mean. An asterisk indicates a significant difference between preferred mates and non-preferred mates ($p<.05$ independent *t*-tests).

	Number of mounts (M)	Number of intros (I)	Number of ejacs (E)	Hit rate (M/I + E) × 100	Mount latency (s)	Intromission latency (s)	Ejaculation latency (s)	III (s)	Number of pups	Testes-to-body-weight ratio	Testosterone-to-creatinine ratio (ng/mg)
Preferred n = 15	6.4 ± 1.9	7.1 ± 1.2*	1.9 ± 0.4*	62.9 ± 7.2	110.9 ± 57.3	71.1 ± 18.3*	502.9 ± 108.6*	117.6 ± 35.7*	4.1 ± 1.1	0.63 ± 0.02	1.72 ± 0.19
Non-preferred n = 15	2.9 ± 0.6	2.9 ± 0.3	0.9 ± 0.1	62.3 ± 6.6	371.8 ± 224.0	515.1 ± 205.4	1628.0 ± 295.5	533.9 ± 181.5	5.9 ± 1.1	0.60 ± 0.02	1.70 ± 0.14

represents the vagaries of paced mating behavior across different cohorts of naturally cycling female rats.

Similar to our recent findings, one male in each pair was preferred by the majority of all females that mated with him across multiple tests [6], and one male in each pair sired significantly more pups than the other [17]. Surprisingly, non-attractive males (preferred by approximately 24% of the females) sired significantly ***more*** pups than attractive males (preferred by approximately 76% of the females) indicating that the non-attractive males had a reproductive advantage.

In general, non-attractive males will have less opportunity to mate (if females spend less time with them); therefore, it is interesting that non-attractive males were more likely to sire offspring. Non-attractive male rats may compensate for limited access to females with altered post-copulatory sexual selection (i.e., sperm competition). Sperm competition could vary between males in terms of 1) the quantity of sperm produced [14], 2) the storage capacity of sperm [34], 3) the ability of sperm to defend against competing males' sperm [34], and 4) the ability to transfer sperm effectively (i.e., by the correct placement of plug or vitality of sperm) [18]. Although each male's capacity to engage in sperm competition was not assessed directly, a positive correlation between testes weight and offspring sired was observed, suggesting that male rats with larger testes sired more offspring. Stockley and Preston [18] found a positive correlation between testes size and sperm production in rats, which would be consistent with siring more offspring. The relationship between heavier testes and siring more offspring could be evidence of greater sperm production or storage between individual rats.

Surprisingly, the results of the present study indicate that male rats with a reproductive advantage possess qualities that female rats tend to find less attractive than males without a reproductive advantage. Female rats could find the non-attractive males more aversive than attractive males. Sexual motivation is diminished in female rats when mating stimulation is perceived as more intense, such as when the medial preoptic area of the hypothalamus is damaged or when genital blood flow is increased following the administration of the phosphodiesterase type-5 inhibitor, zaprinast [35–38]. Therefore, non-attractive males could be more forceful or deliver more intense sexual stimulations than attractive males. This increased forcefulness or intensity could be the result of stronger penile reflexes or deeper penetrations. Strong penile reflexes represent a mechanism by which males can overcome obstacles during sperm competition because these reflexes ensure proper placement of a male's own sperm plug in the female genital tract. Proper placement of sperm plugs that are tight-fitting enough to prevent leakage promote maximal cervical sperm transport, and resist being dislodged by other male's intromissions [39–41]. Therefore, the non-attractive males may be more adept at 1) placing their own sperm plugs and/or 2) removing previously placed sperm plugs, both of which could enhance their reproductive success but could be perceived as more aversive to the female.

Urinary testosterone levels were similar between the group of rats that sired the majority of pups and the group that sired few or none of the pups. Testosterone levels also failed to differentiate attractive males from non-attractive males. There are a number of possible explanations for why testosterone levels did not differentiate between males with a reproductive advantage and those without a reproductive advantage. Although basal testosterone levels may be related to secondary sexual characteristics, and thus, may indicate fitness [7,10], there can be a disconnect between testosterone levels and measures of fertility [42]. Specifically, spermatogenesis may be independent from basal testosterone levels. For example, knocking out the TAF4b gene in male mice impairs spermatogenesis while leaving basal serum testosterone levels unaffected [43]. Furthermore, impaired spermatogenesis in TAF4b knockout mice is also associated with smaller testes size when compared to wild type mice. This finding supports the conclusion that testes size can be related to reproductive success (possibly spermatogenesis), independent of testosterone levels.

Significant variation in testosterone levels among males has been well documented in species that have breeding seasons [24,25,44]. However, in rats, a species that mates continuously throughout the year, testosterone levels are relatively stable with only typical mammalian diurnal fluctuations [45]. Therefore, it is also possible that male Long–Evans rats do not have sufficient inter-individual variation in urinary testosterone levels to distinguish males from one another. In fact, there are very few published reports investigating baseline testosterone levels or individual differences in basal testosterone among males in the literature. One study found very low levels of variation in basal plasma testosterone levels between male mice bred for high levels of wheel running and control lines [46]. Because testosterone concentration in the testes is approximately 40 times higher than in serum [47], studies are currently underway to investigate individual differences between males using intra-testicular testosterone levels.

Although levels of testosterone were not different between groups of males in the current study (i.e., attractive vs. non-attractive; reproductive advantage vs. no reproductive advantage; etc.), measuring testosterone in urine instead of in serum is nonetheless advantageous because collection is non-invasive and reflects androgen production over long periods of time instead of just short-term fluctuations in secretion [48]. Measuring urinary testosterone could be useful in situations where a manipulation is expected to induce significant hormonal alteration. Although much work [48] has been done measuring testosterone in serum, to our knowledge, the profile of basal levels of testosterone excretion in urine has not been well characterized in rats. Of the few studies that have measured urinary testosterone in rats, there are differences in methodologies such as urine collection, type of assay, pretreatment of urine, and variations in cross-reactivity of the antibody. For example, some studies report concentrations of testosterone based on cumulative excretion of testosterone over 24 h, whereas others report concentrations of testosterone excreted on an hourly basis. Nevertheless, our testosterone values are comparable to the range of concentrations reported in other studies [49,50].

Previous research has indicated that the timing between ejaculations is important for determining which male fertilizes more ova, when multiple male rats are mating with a single female rat [39]. For instance, the second male of a pair to ejaculate tends to sire more pups if he ejaculates within a few minutes of the first male [39]. However, if the second male takes longer than 5 min to ejaculate, his advantage is lost and the first male generally sires more pups [39]. The likely explanation for the second male's advantage is strong penile reflexes being able to remove a previous male's sperm plug from the female's genital tract with a single intromission, but only if attempted within 2 to 5 min of the previous male's ejaculation [39,51]. Because, in the current study none of the males ejaculating second did so within 5 min of the first male, it could be predicted that all of the males who ejaculated first should have a reproductive advantage. However, similar to the results of Zewail-Foote et al. [17], the present study failed to confirm this prediction. As discussed by Zewail-Foote et al. [17], methodological differences could explain the inconsistent results between studies. For example, the procedure used in the current study involves simultaneous mating with multiple males, whereas Coria-Avila and colleagues [39] mated females with two males sequentially. This methodological difference could explain the difference in results because, unlike simultaneous mating with multiple males, sequential mating does not result in optimal conditions for sperm competition to occur.

Consistent with the pattern of results reported by Ferreira-Nuño and colleagues [16], non-preferred mates in the present study

displayed fewer intromissions prior to ejaculation. Ferreira-Nuño et al., [16] also reported that non-preferred male rats mating in competition with three other males copulated more quickly than preferred mates, displaying shorter latencies to mount the female and shorter, but not significantly shorter, intromission and ejaculation latencies as well. Although the latency to achieve their first intromission or ejaculation was longer for non-preferred mates than preferred mates in our study, non-preferred mates were able to achieve an ejaculation in less time (i.e., considering that female rats spent 75% less time with the non-preferred mates). Ferreira-Nuño and colleagues [16] concluded that when non-preferred mates experience stress from competition with other males, they are able to ejaculate by mating more quickly and more precisely than the preferred mates.

In conclusion, the results of the present study are important for understanding the characteristics of reproductive success because they support many of the behavioral and physiological findings of Lovell et al. [6] and Zewail-Foote et al. [17] and narrow the scope of a mechanism for these findings. For example, female rats behave differently with their preferred mate and their non-preferred mate. Some males are more attractive to females than others. In addition, when sperm competition can take place, one male in a pair sires the majority of the pups in a litter and this reproductive advantage is related to the overall level of attractiveness of the male. In particular, males that are generally not preferred during most mate choice tests (non-attractive males) have a reproductive advantage, siring significantly more pups than those males that different cohorts of females consistently prefer (attractive males). The present study also investigated the relationship between basal urinary testosterone levels and various behavioral (e.g., copulatory behaviors) and physiological measures (e.g., reproductive success, testes-to-body-weight ratios). Although there were no clear differences between basal urinary testosterone levels of male rats that sired most of the pups and males that sired few or no pups, wet testes weight was positively correlated with the number of offspring sired. Individual differences in sperm competition and/or other physiological measures (e.g., penile reflexes, sperm motility) may explain how non-attractive male rats compensate when access to a female is limited by female mate choice.

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References

[1] Calhoun JB. The ecology and sociology of the Norway rat. Bethesda: U.S. Depart. of Health, Education and Welfare, Public Health Service; 1962.
[2] McClintock MK, Adler NT. The role of the female during copulation in wild and domestic Norway rats (*Rattus norvegicus*). Behaviour 1977;67:67–96.
[3] Robitaille JA, Bovet J. Field observations on the social behaviour of the Norway rat (*Rattus norvegicus*). Biol Behav 1976;1:289–308.
[4] Erskine MS. Solicitation behavior in the estrous female rat: a review. Horm Behav 1989;23:473–502.
[5] Coopersmith C, Erskine MS. Influence of paced mating and number of intromissions on fertility in the laboratory rat. J Reprod Fertil 1994;102:451–8.
[6] Lovell JL, Diehl A, Joyce E, Cohn J, Lopez J, Guarraci FA. "Some guys have all the luck": mate preference influences paced-mating behavior in female rats. Physiol Behav 2007;90:537–44.
[7] Drickamer LC, Gowaty PA, Holmes CM. Free female mate choice in house mice affects reproductive success and offspring viability and performance. Anim Behav 2000;59:371–8.
[8] Zala SM, Chan BK, Bilbo SD, Potts WK, Nelson RJ, Penn DJ. Genetic resistance to infection influences a male's sexual attractiveness and modulation of testosterone. Brain Behav Immun 2008;22:381–7.
[9] Andersson M, Simmons LW. Sexual selection and mate choice. Trends Ecol Evol 2006;21:296–302.
[10] Preston BT, Stevenson IR, Pemberton JM, Coltman DW, Wilson K. Overt and covert competition in a promiscuous mammal: the importance of weaponry and testes size to male reproductive success. Proc R Soc 2003;270:633–40.
[11] Bangham J, Chapman T, Partridge L. Effects of body size, accessory gland and testis size on pre- and postcopulatory success in *Drosophila melanogaster*. Anim Behav 2002;64:915–21.
[12] Sachser N, Schwarz-Weig E, Keil A, Epplen JT. Behavioural strategies, testis size, and reproductive success in two caviomorph rodents with different mating systems. Behaviour 1999;136:1203–17.
[13] Hinson ER, Hannah MF, Norris DE, Glass GE, Klein SL. Social status does not predict responses to Seoul virus infection or reproductive success among male Norway rats. Brain Behav Immun 2006;20:182–90.
[14] Ramm SA, Parker GA, Stockley P. Sperm competition and the evolution of male reproductive anatomy in rodents. Proc R Soc 2005;272:949–55.
[15] Birkhead TR, Pizzari T. Evolution of sex: postcopulatory sexual selection. Nat Rev Genet 2002;3:262–73.
[16] Ferreira-Nuño A. Copulatory pattern of male rats in a multiple partner choice arena. J Sex Med 2010;7(12):3845–56.
[17] Zewail-Foote M, Diehl A, Benson A, Lee KH, Guarraci FA. Reproductive success and mate choice in Long–Evans rats. Physiol Behav 2009;96:98–103.
[18] Stockley P, Preston BT. Sperm competition and diversity in rodent copulatory behaviour. J Evol Biol 2004;17:1048–57.
[19] delBarco-Trillo J, Ferkin MH. Male mammals respond to a risk of sperm competition conveyed by odours of conspecific males. Nature 2004;431:446–9.
[20] Pound N, Gage MJG. Prudent sperm allocation in Norway rats, *Rattus norvegicus*: a mammalian model of adaptive ejaculate adjustment. Anim Behav 2004;68:819–23.
[21] Vaughn AA, Delbarco-Trillo J, Ferkin MH. Sperm investment in male meadow voles is affected by the condition of the nearby male conspecifics. Behav Ecol 2008;19:1159–64.
[22] Nyby JG. Reflexive testosterone release: a model system for studying the nongenomic effects of testosterone upon male behavior. Front Neuroendocrinol 2008;29:199–210.
[23] Taylor GT, Weiss J, Pitha J. Testosterone in a cyclodextrin-containing formulation: behavioral and physiological effects of episode-like pulses in rats. Pharmacol Res 1989;6:641–6.
[24] Garamszegi LZ, Eens M, Hurtrez-Bousses S, Moller AP. Testosterone, testes size, and mating success in birds: a comparative study. Horm Behav 2005;47:389–409.
[25] Malo AF, Roldan ER, Garde JJ, Soler AJ, Vicente J, Gortazar C, et al. What does testosterone do for red deer males? Proc R Soc 2009;276:971–80.
[26] Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: some helpful considerations. Braz J Biol 2002;62:609–14.
[27] Zipse LR, Brandling-Bennett EM, Clark AS. Paced mating behavior in the naturally cycling and the hormone-treated female rat. Physiol Behav 2000;70:205–9.
[28] Hardy DF, DeBold JF. Effects of coital stimulation upon behavior of the female rat. J Comp Physiol Psychol 1972;78:400–8.
[29] Burn CC, Mason GJ. Effects of cage-cleaning frequency on laboratory rat — reproduction, cannibalism, and welfare. Appl Anim Behav Sci 2008;114:235–47.
[30] DeSantis DT, Schmaltz LW. The mother–litter relationship in developmental rat studies: cannibalism vs caring. Dev Psychobiol 1984;17:255–62.
[31] Dixson AF, Anderson MJ. Sexual behavior, reproductive physiology and sperm competition in male mammals. Physiol Behav 2004;83:361–71.
[32] Ferreira-Nuño A, Morales-Otal A, Paredes RG, Velazquez-Moctezuma J. Sexual behavior of female rats in a multiple-partner preference test. Horm Behav 2005;47:290–6.
[33] Beach FA. Sexual attractivity, proceptivity, and receptivity in female mammals. Horm Behav 1976;7:105–38.
[34] Hosken D, Stockley P. Sexual selection and genital evolution. Trends Ecol Evol 2004;19:87–93.
[35] Clark AS, Meerts SH, Guarraci FA. Zaprinast, a phosphodiesterase type-5 inhibitor, alters paced mating behavior in female rats. Physiol Behav 2009;96:289–93.
[36] Guarraci FA, Clark AS. Ibotenic acid lesions of the medial preoptic area disrupt the expression of partner preference in sexually receptive female rats. Brain Res 2006;1076:163–70.
[37] Guarraci FA, Megroz AB, Clark AS. Paced mating behavior in the female rat following lesions of three regions responsive to vaginocervical stimulation. Brain Res 2004;999:40–52.
[38] Yang LY, Clemens LG. MPOA lesions affect female pacing of copulation in rats. Behav Neurosci 2000;114:1191–202.
[39] Coria-Avila GA, Pfaus JG, Hernandez ME, Manzo J, Pacheco P. Timing between ejaculations changes paternity success. Physiol Behav 2004;80:733–7.
[40] McClintock MK, Toner JP, Adler NT, Anisko JJ. Postejaculatory quiescence in female and male rats: consequences for sperm transport during group mating. J Comp Physiol Psychol 1982;96:268–77.
[41] O'Hanlon JK, Sachs BD. Fertility of mating in rats (*Rattus norvegicus*): contributions of androgen-dependent morphology and actions of the penis. J Comp Psychol 1986;100:178–87.
[42] Crews D. Gamete production, sex hormone secretion, and mating behavior uncoupled. Horm Behav 1984;18:22–8.
[43] Falender AE, Freiman RN, Geles KG, Lo KC, Hwang K, Lamb DJ, et al. Maintenance of spermatogenesis requires TAF4b, a gonad-specific subunit of TFIID. Genes Dev 2005;19:794–803.
[44] Kempenaers B, Peters A, Foerster K. Sources of individual variation in plasma testosterone levels. Philos Trans R Soc 2007;363:1711–23.
[45] Baum MJ. Sexual behavior in the male. In: Becker J, Breedlove M, Crews D, McCarthy MM, editors. Behavioral Endocrinology. Cambridge, Massachusetts: MIT Press; 2002. p. 159–203.
[46] Klomberg K, Garland T, Swallow J, Carte P. Dominance, plasma testosterone levels and testis size in house mice artificially selected for high levels. Physiol Behav 2002;77:27–38.

[47] McLachlan RI, Wreford NG, O'Donnell L, de Krester DM, Robertson DM. The endocrine regulation of spermatogenesis: independent roles of testosterone and FSH. J Endocrinol 1996;148:1–9.
[48] Venturelli E, Cavalleri A, Secreto G. Methods for urinary testosterone analysis. J Chromatogr B Biomed Appl 1995;671:363–80.
[49] Ortiz RM, Wade CE, Morey-Holton E. Urinary excretion of LH and testosterone from male rats during exposure to increased gravity: post-spaceflight and centrifugation. Proc Soc Exp Biol Med 2000;225:98–102.
[50] Yokoi K, Uthus EO, Nielsen FH. Nickel deficiency diminishes sperm quantity and movement in rats. Biol Trace Elem Res 2003;93:141–53.
[51] McClintock MK, Anisko JJ, Adler NT. Group mating among Norway rats: II. The social dynamics of copulation: competition, cooperation and mate choice. Anim Behav 1982;30:410–25.