Paper 4

Sociosexual behaviors and reproductive success of rats (*Rattus norvegicus*) in a seminatural environment

Sociosexual Behaviors and Reproductive Success of Rats (*Rattus norvegicus*) in a Seminatural Environment

Xi Chu[1]*, Fay A. Guarraci[2], Anders Ågmo[1]

[1]Department of Psychology, University of Tromsø, Tromsø, Norway

[2]Psychology Department, Southwestern University, Texas, USA

*Corresponding author

E-mail address: xi.chu@uit.no (XC)

Abstract

A promiscuous pattern of copulation has been reported in both wild and domestic rats, and multiple paternity is common. In the present study we determined whether male sociosexual behaviors were associated with reproductive success or not. Groups of rats (3 males and 4 cycling females) were housed in a seminatural environment for a period of 8 days. Sociosexual interactions were observed whenever one or several females were in behavioral estrus. Paternity of the offspring sired was determined by analyses of single nucleotide polymorphisms. Fertility was quantified either as total number of offspring, or the mean proportion of offspring from all females, sired by the male. Although the total numbers of male social and sexual behaviors during the 8 days period was unrelated to reproductive success, male fertility was correlated with the number of intromissions and ejaculations. In addition intromission and ejaculation ratios (the proportion of mounts ending in intromission and the proportion of the total number of ejaculations received by a female that was contributed by a particular male, respectively) were also correlated with male fertility. Fewer mounts as well as fewer male pursuits of female were demonstrated by males siring entire litters than by males siring multipaternity litters. Ejaculation order was unrelated to fertility. Male or female preferences (based on various sociosexual interactions) were not strongly related to fertility. Female preference only showed a limited effect. Specifically, preferred males (males who were sniffed most) sired fewer pups. Male dominance status did not affect female preferences, copulatory behavior or fertility. It appears that only behaviors directly related to sperm transfer are important for fertility. Social behaviors both in males and females play a very limited or no role in determining rats' fertility.

Introduction

In wild rats, females mate with several males during the period of estrus, and males may simultaneously mate with several females [1,2]. Mating continues as long as there are females remaining in estrus [1-4]. This is also the case in domestic rats housed in a seminatural environment [5,6]. Whenever two or more females have overlapping behavioral estrus, male rats may sire offspring with multiple females within a short period. Whenever more than one sexually active male is available, females may carry offspring from several males in the same litter. Multiple paternity has been described both in wild and domestic rats [7-9].

The factors determining male rat reproductive success are poorly known, but several hypotheses have been put forth. Intrinsic characteristics of the male, for example dominance or sperm quality [10,11], have been proposed to be associated with high fertility. Circumstantial factors like ejaculate order or the number of ejaculations achieved with a particular female [12] have also been suggested to be important to male fertility. The male's attractiveness to females may be another potential determinant of reproductive success. It has been surmised that females prefer to copulate with highly attractive males rather than with less attractive males, and it has been assumed that male attractiveness is associated with favorable, heritable characteristics [13]. In support of this notion, it has been reported that preferred male house mice have a reproductive advantage over non-preferred mice [14]. Dominance has also been considered to be associated with reproductive advantage. A dominant male rat is more attractive to females than a subordinate and a subordinate male's mating behavior may be inhibited by the dominant male [15-17]

In rats, experimental studies of relative male reproductive success have used a design in which two males were allowed to copulate with the same female either simultaneously or in rapid succession [e.g. 18-22]. The duration of mating was always quite short. This is rather

different from copulatory interactions in the wild, and the external validity of the laboratory studies is probably limited. Data from a seminatural environment, in which mixed sex groups live together for some time, seem to offer an excellent opportunity to test some of the hypotheses concerning male rat reproductive success with larger external validity. Here we present data on sociosexual interactions during the entire period of each female's behavioral estrus in groups of 4 females and 3 males living in a seminatural environment. Paternity for pups sired during the study was determined by analyses of single nucleotide polymorphisms (SNPs). The role of ejaculate order, characteristics of male and female sexual behaviors, and male and female mate preferences in male reproductive success were investigated. We also evaluated the potential role of dominance for sexual behaviors and fertility.

Materials and Methods

The study has been approved by the Norwegian National Animal Research Authority.

Subjects

Male (300 g upon arrival) and female (250 g on arrival) Wistar rats were purchased from Charles River WIGA (Sulzfeld, Germany). The animals were housed in same sex pairs in Macrolon® IV cages in a room with controlled temperature (21 ± 1 °C) and humidity (55 ± 10 %) and a 12:12 h light/dark cycle (lights on 0800). Commercial rat pellets and tap water were provided ad libitum.

Apparatus

The seminatural environment used in this study has been described in detail in [5,6]. Briefly, it measured 2.8 x 2.4 m and consisted of a complex burrow system and an open area.

There were 4 small openings (8 x 8 cm) between the burrow and the open area. A light-blocking wall of extruded polyethylene foam was used to divide the room in which the environment was installed into two parts, thereby providing the possibility to vary the light intensity in the open area while maintaining the burrow in complete darkness. Video cameras were centered above the open area and the burrow, respectively.

Procedure

The subjects were marked shortly before they were introduced into the seminatural environment. They were shaved in different areas of the back and their tails were marked with different numbers of black stripes. Before a group of animals was introduced into the seminatural environment, the floor was covered with 2 cm of aspen wood shavings. A few wood sticks and 3 plastic shelter huts were provided in the open area, and nest building material was put in the nest boxes. About 3 kg of food pellets were provided in a corner of the open area, and 4 water bottles were freely accessible in that corner. The 12:12 h light/dark cycle was preserved in the open area. During the dark phase, light intensity was about 1 lx at floor level. It was about 180 lx during the light phase. The burrow was maintained in total darkness for the rats but illuminated with 2 infrared lamps for the video camera. The video recorders were activated when introducing the animals at 13:00 on Day 0. Recording was then continuous for a period of 8 days. Additional procedural details can be found in [5,6].

The females were housed individually for 3 weeks after the end of the observation period, and carefully examined for pregnancy every 2 or 3 days. Pregnant females were given nest material, and then left undisturbed until birth of the pups. The number of the pups was determined as soon as possible after birth. Tissue samples for paternity test were collected 25 - 30 days after pups were born.

Design

Five groups were used. Each group consisted of 4 females and 3 males. All subjects were intact and sexually naïve. Subjects in the same group came from different cages to ensure that they were unknown to each other at the beginning of observation. In the period of 8 days, each female would enter into estrus at least once.

Behavioral observations

From the video record, we observed the duration and/or the frequency of the behaviors defined in Table 1. The animal that initiated the behavior and the recipient of the behavior were also recorded in order to make it possible to determine the amount of interaction between specific individuals. The Observer XT 10 (Noldus, Wageningen, Netherlands) was used for all observations. Behavior was recorded during the entire period of behavioral estrus for each of the females. In case a female entered into estrus twice, only the second estrus was considered, since the first must have been infertile. Behavioral estrus started when a female first displayed a lordosis in response to a mount and continued until there was an interval of more than 60 min after a lordosis without any further sexual activity. The moment of that lordosis was considered to be the end of behavioral estrus.

In the seminatural environment, male sexual behavior is better described in terms of copulatory bouts than in terms of the often used ejaculatory series (see [6]. A copulatory bout is defined as the interval between a male's first mount and the beginning of a period of sexual inactivity lasting for more than 60 min. Males may have several bouts during one female's behavioral estrus. The total duration of a male's bouts is the time he was engaged in sexual activity. In addition to recording the behaviors shown in Table 1, we determined postejaculatory interval (time between ejaculation and the following intromission regardless of whether it occurred with the same or another female), copulatory rate (the number of

mounts + the number of intromissions performed with all females during a bout divided by bout length) and intromission ratio (the number of intromissions divided by the number of mounts + number of intromissions). Furthermore, we determined the proportion of all ejaculations received by a female coming from a particular male, expressed as a percentage ((number of ejaculations received from a particular male / total number of ejaculations received) x 100). This percentage is called ejaculation ratio.

To determine dominant - subordinate relationships among the 3 males in each group we analyzed fighting with or fleeing from another male as dominant and submissive behavior, respectively. However, fighting among the males occurred very rarely. The duration of fighting was only 6.7 + 3.2 s (mean + SE, N = 15) and the frequency was 1.8 + 0.7 times. These numbers were too low to allow for any meaningful analysis. Therefore we used the number of flights (mean 89.33 + 23.06 times) to determine submission, which we considered as opposite to dominance. Rats generally flee from others in two situations: 1) One rat (the loser) runs away from another (the winner) very quickly immediately after contact, sometimes even before contact. 2) After an episode of nose – off or boxing, one rat (the loser) suddenly runs away.

Within each group, we first compared the males' flight frequency between all possible pairs of males. The dominant male in a pair was the one with the lowest number of flights. The dominance rank was then decided by the comparison of pairs as described by [23].

Tissue collection for DNA extraction and paternity identification

Pinnae snips were collected from mother rats, potential father rats and all 156 pups generated during the experiment. A portion of pinna (approximately 5 x 5 mm) was cut off by clean, sharp scissors from an ear immediately after euthanasia. The samples were stored at −20 °C before they were shipped for analysis. DNA was extracted from the tissue samples for

single nucleotide polymorphism (SNP) analysis. Paternity determinations were performed by Harlan Laboratories (Indianapolis, Indiana, USA). First, mothers and potential fathers were tested on 48 SNP markers in order to evaluate the feasibility of unequivocal identification of fathers. Each pup was then tested on those markers. It turned out that fatherhood could not be unequivocally determined for 16 % of the pups (25 out of 156).

Data preparation and statistics

Each of the 20 females used in this study had the opportunity to copulate with 3 males. Thus, potentially there are a total of 60 pairs. Even though each of the females was repeated in 3 pairs, we considered our observations as independent because we used a pair rather than an individual animal as the experimental unit. Male and female sociosexual behaviors within each pair were calculated either as total duration or total frequency of each behavior directed towards the male and female partner, respectively. We also determined the number of offspring sired by the male in each pair as well as the proportion of the female's offspring sired by that male.

For the analysis of the role of dominance, we compared behavior and fertility in the dominant and subordinate males, ignoring the male intermediate between the two. The importance of male preference for a particular female was determined by comparing the preferred female with the non-preferred female according to several criteria. In case the male could choose between more than two females, those intermediate between the most preferred and the least preferred were ignored. For evaluating the role of female preference for a particular male, several criteria for preference were used and the preferred male according to each of the criteria was compared to the non-preferred male, whereas the intermediate male was ignored.

The majority of the data failed to follow a normal distribution according to the Shapiro-Wilk test. Therefore, nonparametric tests were employed to determine the statistical significance of these data. The Spearman correlation was used to evaluate associations between items of sociosexual behavior and fertility, and the Mann-Whitney U test was used for the evaluation of independent measures. Proportions, like the intromission and ejaculation ratio, were normally distributed. They were, consequently, analyzed with parametric procedures, that is the Pearson correlation or the *t*-test. All data in the text and figures are mean + standard error of the mean, and all probabilities given are two-tailed.

Results

General description

All 15 males in this study copulated and ejaculated at least once. Of the 20 females three did not enter estrus during the observation period and one female did not get pregnant. Two females cannibalized their pups shortly after birth, making it impossible to determine the number or the paternity of the offspring. Thus, paternity data were obtained from 14 females. Consequently, there were 42 male – female pairs. In 8 of these pairs the paternity test was unable to identify the father of some of the pups, leaving 34 pairs in which paternity was successfully determined for all pups. These pairs involved 14 females and 13 males.

The relationship between sociosexual behavior within the pair and fertility

We divided the 34 pairs with successfully determined paternity into two groups depending on the male's reproductive success, the Offspring group (pairs with pups, N = 14)

and the Non-offspring group (pairs with no pups, N = 20). Neither the number of copulatory bouts nor the duration of these bouts differed between the two groups according to the Mann – Whitney *U* test (*p*s > 0.41). In contrast, the number of copulatory acts displayed by the males in the Offspring group was significantly greater than that displayed by the Non-offspring group, specifically for the number of intromissions ($U = 62$, $p = 0.006$) and ejaculations ($U = 61$, $p = 0.005$) but not for the number of mounts ($U = 115.5$, $p = 0.391$). The data are illustrated in Fig. 1 A. Additionally, males in the Offspring group demonstrated a higher ejaculation ratio (number of ejaculations received from the within-pair male / total number of ejaculations received) than the males in the Non-offspring group (49.5 + 6.1 % vs. 22.2 + 5.6 %, $t(32) = 3.244$, $p = 0.003$), whereas the difference in intromission ratio was not statistically significant (0.41 + 0.06 and 0.26 + 0.26, respectively, $t(32) = 1.697$, $p = 0.099$). We found no other statistically significant differences between groups in terms of male or female sociosexual behavior (*p*s > 0.20, Fig. 1 A and B).

The data reported in the preceding paragraph show that the intensity of some male sexual behavior patterns, i.e. intromissions and ejaculations, as well as the proportion of all ejaculations received by the female, are important for a male's likelihood of producing offspring within a pair.

Whether the *number* of offspring sired is related to sociosexual behavior or not is an entirely different question. In order to answer that question, we calculated the Spearman correlation between number of offspring or the proportion of offspring sired by the male within the pair and male sociosexual behaviors. As shown in Table 2, the number of offspring and the proportion of pups sired were highly correlated. The number of intromissions and ejaculations as well as the intromission and ejaculation ratios was correlated with both the number of pups and the proportion of pups sired. However, there was no relationship between the number of mounts and fertility. Likewise, there was no association between male pursuit

of the female, sniffing or anogenital sniffing of the female and male fertility. Furthermore, none of the female behaviors listed in Table 2 was related to male within-pair fertility. It seems, then, that the likelihood of siring offspring and the number of offspring sired with a particular female both are determined by the aspects of male behavior towards that female that are directly associated with sperm transfer. Intromissions are needed for triggering ejaculation, the intromission ratio shows the male's capacity of ending a mount with vaginal penetration, and the number of ejaculations determines the amount of sperm transferred to the female. Moreover, the ejaculation ratio determines the proportion of the total number of sperm inseminated that was contributed by the within-pair male.

Several males displaying ejaculation with their pair-partner did not sire any offspring at all with the partner. In order to find possible behavioral differences between these males and those fathering offspring we compared the pairs with male offspring (the Offspring group, N = 14) with those without but in which the male had ejaculated at least once with the within-pair female (the Non-offspring ejaculation group, N = 11). There was no difference in any sociosexual behavior (*p*s > 0.09, data not shown). Furthermore, the number of ejaculations with other females (extra-pair ejaculations) performed by the males before their first ejaculation within the pair did not differ between the Offspring and Non-offspring ejaculation groups (1.64 + 0.63 and 3.09 + 0.72, respectively, $U = 51.5$, $p = 0.141$). Likewise, the number of extra-pair ejaculations received by the female before the first within-pair ejaculation was similar in both groups (1.36 + 0.49 vs. 1.36 + 0.54, $U = 74.5$, $p = 0.882$).

Multiple vs. single paternity

Among the 14 pairs with offspring and with certain paternity identification, only 6 revealed single paternity. The offspring of the remaining 8 pairs showed multipaternity. We divided the 14 pairs into 2 groups according to whether the male in the pair sired the entire

litter or not. When comparing the Multiple (N = 8) and Single (N = 6) paternity groups, we found that the males in the Multiple paternity group displayed more mounts ($U = 4.5$, $p = 0.01$) and more pursuit of the female ($U = 4.0$, $p = 0.01$; Fig. 2) than the males in the Single paternity group. No difference between these groups was found in the number of intromissions ($U = 13.5$, $p = 0.17$) or ejaculations ($U = 20.5$, $p = 0.63$) performed by the pair partner. Likewise, the partner's ejaculation ratio did not differ between the Multiple and Single paternity groups (46.4 + 6.0 % vs. 53.5 + 12.4 %, $t(12) = 0.561$, $p = 0.585$).

It is possible that single or multiple paternity depends on the other males' behavior towards the female in the pair. For example, the interval between the pair-male's ejaculation and ejaculation by another male could be important. In the Single paternity group, this interval was 64.2 + 32.6 min and in the Multiple paternity group it was 49.9 + 16.9 min. The difference between groups was not significant ($U = 11$, $p = 0.831$). We also examined the interval between the pair-male's ejaculation and the next intromission the female received. This interval was 15.0 + 4.9 min in the Single paternity group and 29.6 + 21.4 min in the Multiple paternity group. The group difference was not significant ($U = 3$, $p = 0.317$).

The role of ejaculation order

In the pairs in which the pair mate was the first to ejaculate with the female, this male sired 4.8 ± 1.4 pups (N = 13) with that female. When the pair mate was the second to ejaculate with the female, he sired 4.9 ± 1.5 pups (N = 10). The difference is non-significant ($U = 62.5$, $p = 0.87$). There were 6 pairs in which the female received ejaculations from all 3 males. Unfortunately, the SNP structure of the offspring in 4 of these pairs did not allow for unambiguous determination of paternity in all pups. However, it was possible to determine a minimum and a maximum number of pups that could have been sired by each male in the 4 females involved. When the pair mate was the 3rd male ejaculating with the partner he did not

sire any pups at all in the 2 females with known paternity for all the offspring. In the 4 other females, the 3rd male ejaculating sired either between 1 and 4 pups (minimum and maximum), or between 0 and 8 pups (two males), or between 9 and 15 pups, respectively. Taking the minimum numbers of offspring sired, the male ejaculating 3rd sired a mean of 1.67 +1.48 pups, whereas the maximum number gives a mean of 5.83 + 2.34 pups. Comparing the number of pups produced by the male ejaculating 1st and 3rd using the minimum number for the latter shows that there was no significant difference ($U = 27$, $p = 0.255$). This was also the case when the maximum number of pups was used for comparison ($U = 35.5$, $p = 0.749$).

In order to further evaluate the role of ejaculation order we compared the number of offspring sired by the male achieving the first versus the male achieving the last ejaculation with a female. Thus, here the analysis is based on ejaculation order without any consideration of male – female pairs. If the same male was both first and last he was excluded from analysis. There were 8 females that received their first and last ejaculation from different males. The first male sired 5.6 + 1.9 pups and the last male sired 4.1 + 1.5 pups ($U = 26.5$, N = 8, $p = 0.552$). The proportion of 1st males siring any offspring at all was 47.9 + 15.7 %, and that of the last male was 39.6 + 15.2 % ($t(14) = 0.38$, $p = 0.712$). These data show that there is no significant advantage for the male that ejaculated first.

Male preferences and fertility

In order for a male to show preference for a certain female he needs to have the possibility of choice between at least two estrous females. In one of our groups, 3 females had overlapping estrus, and in another two groups there were 2 females with overlapping estrus. This means that we could determine preference for a total of 9 males. Unfortunately, the fertility could not be determined for 3 males because of a mother's cannibalism or failed SNP analysis.

Preference for a particular female can be defined in many, not necessarily coinciding, ways. There may be a preferred female for copulation and pursuit, whereas there is another female preferred for sniffing and/or anogenital sniffing, for example. Consequently, we determined several male preferences based on different behaviors. First we determined a male's preferred mate as the female the male achieved the most copulatory behaviors with. When the number of copulatory acts was used as criterion for determining preference, the females were first ranked according to the number of ejaculations received from the male. In case of ties, the female receiving the largest number of intromissions was assigned the higher rank. In case the tie persisted, the number of mounts was taken into account. The males did not sire more offspring with their preferred female than with their non-preferred (3.8 + 2.5 vs. 3.3 + 2.1, $z = 1.00$, N = 6, $p = 0.317$).

The determination of male preference was then based on the amount of pursuit, sniffing or anogenital sniffing of the female. The female being most pursued, most sniffed or anogenitally sniffed was considered as the preferred female whereas the one least pursued, least sniffed or anogenitally sniffed was considered the avoided female. Male behavior towards preferred and avoided females was compared. There was no difference between preferred and avoided females (data not shown, ps > 0.25) regardless of which of the 3 preference criteria that was used. These data suggest that male preference for a certain female, either based on copulatory activity or on affiliative behaviors, has no consequence for fertility.

Female preferences and fertility

Each female copulated with all 3 males during her behavioral estrus. However, the amount of copulatory behavior displayed in response to each of the 3 males varied. Similarly, the estrous female displayed more paracopulatory behaviors in response to, and sniffed and anogenitally sniffed, one male more than the others. All these behaviors can be used to

determine female preference for a particular male. In order to ascertain whether female preference for a particular male affected male fertility, we related the behavioral data of all pairs in which the female partner alone was in estrus to male fertility. Thus, we excluded data from pairs in which female interaction with the male in the pair could have been affected by the presence of other estrous females. The behavioral estrus in six females did not overlap with that of other females. Thus, there were 18 female – male pairs satisfying the criterion. We determined female preference based either on the duration of paracopulatory behavior, the number of lordoses or the duration of sniffing or of anogenital sniffing of each of the males. The male with the longest duration or the largest number was considered the preferred male and the male with the shortest duration or lowest number was considered the non-preferred male.

The number of offspring sired by the preferred male did not differ from that of the non-preferred male when female preference was based on the duration of paracopulaory behavior or the number of lordosis responses (ps > 0.71). However, paracopulatory behavior and the number of lordosis responses displayed are strongly correlated with male pursuit and male mounting [5,6] meaning that they may be determined more by the male than by female preference. Therefore, female sniffing or anogenital sniffing of the male may be more appropriate indicators of female preference, because these behaviors are entirely or mainly initiated by the female herself. However, neither preference based on female sniffing nor anogenital sniffing affected male fertility (ps > 0.14). We also evaluated the role of female avoidance behaviors, i.e. nose – off and rejection. The most avoided male did not differ from the least avoided male in terms of fertility (ps > 0.46).

In 4 of the pairs included in the preceding analysis the male failed to ejaculate. If we limit the analysis of the role of female preferences to the 14 pairs in which the male ejaculated at least once, it turns out that preference based on paracopulatory behavior or the number of

lordosis responses still did not affect the number of offspring sired (ps > 0.40). When female preference was based on sniffing the male, the preferred male sired fewer pups than the non-preferred male (2.3 + 0.8 vs. 7.0 + 1.6 pups, $z = 2.03$, N = 6, $p = 0.042$). No difference in fertility was found when female preference was based on anogenital sniffing, or on the avoidance behaviors of nose – off or rejection (ps > 0.42).

The role of male dominance

In one of the 5 groups used in this study, only one male fled from the others, making it impossible to appropriately determine dominance/submission. Therefore, we could identify only 4 dominant and 4 subordinate males based on flight behavior. To answer the question of whether dominance was important to male copulatory behavior and fertility, we compared the total number of each copulatory act as well as fertility indices between Dominant and Subordinate males. The mean number of copulatory bouts for Dominant males was 3.0 + 1.1, and the number of copulatory bouts for the subordinate was 4.8 + 0.9 ($U = 3.5$, $p = 0.19$). The total duration of copulatory bouts in Dominant and Subordinate males was 9.3 + 3.0 h and 8.4 + 1.1 h, respectively ($U = 7$, $p = 0.77$). When comparing the numbers of copulatory acts performed by the males, no group difference was found (Fig. 3 A, ps > 0.29). Neither the number of pups nor the proportion of pups was affected by dominance (Fig. 3 B, ps > 0.59). In addition, male dominance did not affect female preference, because the duration of female sniffing of and paracopulatory behavior displayed to dominant and subordinate males did not differ (Fig. 3 C, ps > 0.14).

Male sociosexual behavior and fertility

In the earlier sections we mostly analyzed male behavior within pairs. The data in this section are based on male behavior directed towards all females in each group. Two of the 15

males copulated only with the females giving birth to pups for which paternity could not be determined. These males were excluded from analysis. The 13 remaining males ejaculated 4.5 + 0.5 times and fathered a mean number of 7.8 + 2.4 pups during the entire experiment.

Spearman (and Pearson) correlations between the number of offspring or the proportion of offspring and male sociosexual behaviors are shown in Table 3. Neither the number of copulatory acts nor the intensity of these acts, expressed as copulatory rate or the postejaculatory interval, was related to male fertility. Likewise, there was no association between pursuit of the females, sniffing and anogenital sniffing of females and fertility.

The surprising absence of any relationship between the number of intromissions and particularly of ejaculations and fertility prompted us to make a further analysis. We selected the 4 males with the highest number of ejaculations (mean 6.5 + 0.3) and the 7 males with the fewest number (3.3 + 0.4) and compared their fertility. There was no difference neither in the number of pups (6.3 + 4.7 vs. 7.3 + 3.1, $U = 11$, N1 = 4, N2 = 7, $p = 0.562$) nor in the proportion of pups fathered (19.7 + 15.3 % vs. 29.2 + 11.7 %, $t(9) = 0.490$, $p = 0.636$). A similar comparison was made between the 5 males with the largest number of intromissions (53.0 +5.4) and the 5 with the lowest number (21.6 + 3.1). Fertility was similar in both groups of males with regard to the number of pups (5.6 + 3.7 vs. 8.8 +4.1, $U = 9$, N = 5, $p = 0.459$), as well as with regard to the proportion of pups (17.7 + 12.0 % vs. 36.9 + 15.1 %, $t(8) = 0.99$, $p = 0.350$).

Discussion

A most interesting observation was that male reproductive success was related to the intensity of copulatory behavior within the pair. The greater the number of intromissions and ejaculations performed, the greater is the likelihood of producing offspring and the number of offspring produced with that female. It may be reasonable to propose that many ejaculations

with a particular female leads to more transfer of sperm than few ejaculations, thereby assuring that the male contributes a greater proportion of sperm to that female. To the contrary, the overall frequency of copulatory behavior displayed with all females in the group is not related to male fertility. One possible explanation is that a large investment of copulatory activity in one female leads to reduced investment in other females. Thus, the reproductive advantage acquired with the female in which the large investment is made seems to be offset by a reproductive disadvantage with other females. This argument obviously presupposes that the male's sexual capacity is limited. There is, in fact, much evidence that this is the case. In pair tests, a male rat usually ceases to copulate after 7 – 10 ejaculations [24,25]. After that, he is completely unresponsive to a female during at least 24 h and needs two weeks to recover full sexual vigor [e.g. 26]. Furthermore, the sperm content of the ejaculate gets rapidly reduced, and after 5 - 7 ejaculations there is virtually no sperm transferred to the female [27-29]. In the seminatural environment males ceases to copulate after fewer ejaculations than in a pair test [6], suggesting that their copulatory capacity may be more limited. Consequently, intense ejaculatory activity with one female leads to reduced activity with others. If true, this argument means that a male may concentrate his ejaculatory activity to one female, thereby increasing the likelihood of siring some or most of her offspring, or he may dilute his copulatory activity among several females, siring offspring with some of them. A latter kind of male would score low on within-pair fertility without necessarily doing so on total fertility in the group. Such a mechanism could well explain the promiscuous mating strategy common among rats. It must also be noted that the males in the present study employed both mating strategies. When several females were in estrus simultaneously, most males distributed their copulatory activity evenly between them. When the females entered estrus sequentially, most males copulated intensely with some and little

with others. There was no order effect, meaning that the female entering estrus first did not receive more copulatory activity than the female entering last.

Male reproductive success was not dependent on social behaviors such as pursuit of the female, sniffing or anogential sniffing of the female or on the number of mounts when analyzed within pairs or within the whole group. This finding is not unexpected, because neither non-sexual social behaviors nor mounts contribute to sperm transfer. However, females with offspring from multiple fathers were more pursued and mounted by the males, and they displayed more paracopulatory behaviors than females in which there was a single father. It appears, then, that these females were more attractive to the males and/or responded more to the male's approaches. However, the number of intromissions and ejaculations received by the female did not influence multiple versus single paternity. How enhanced attractiveness would enhance the probability of multipaternity without affecting the number of intromissions and ejaculations received cannot be explained at the present.

Previously published results have suggested that some social preferences (as measured in a variety of different ways) are related to reproductive success. For example, in mice, the likelihood that females would produce offspring was found to be superior when mated with a preferred male than when mated to a non-preferred male [14]. Preference was based on the relative time spent in the vicinity of each of two inaccessible males during a 10 min test. Interestingly, there was no difference in the litter size. Similar advantages have been reported of male preference for female mice [30]. Others, however, failed to find any effect on fertility when male mice were allowed to mate with their preferred or non-preferred female [31]. In rats, female preference for one of two males did not affect female [32] or male fertility [22]. Interestingly, males systematically being preferred by female rats sired fewer pups than less attractive males [33]. The present results show that male or female preferences, using a number of different methods for determining preference, do not affect fertility.

Another factor supposed to affect fertility is ejaculation order. However, evidence is mixed. Even though it has been reported that a 2nd male sires more offspring than a 1st male [34], it was later shown that this was the case only when copulation with the 2nd male was initiated immediately following the 1st male's ejaculation [18]. If there was an interval of 5 or 10 min between the two males, it turned out that the 1st male sired more offspring. It should be added that the interval between two successive ejaculations involving the same female always exceeded 10 min in the seminatural environment. However, both studies mentioned above have the disadvantage that paternity determination was achieved by using two strains of males. Strain differences in sperm competitivity might, then, be a confounding variable. When the order of ejaculation is varied between males of the same strain, no order effect is detected [22,33], consistent with the present data.

There were very few agonistic encounters in the seminatural environment. Direct fighting was virtually inexistent, making it impossible to use winner/looser in fights as a basis for determining dominance. Based on fleeing from another male as an indicator of submission or dominance, we found no difference in sexual behavior or attractiveness between dominant and subordinate males. Likewise, dominance did not affect the males' fertility. Similar results have been reported previously [35,36]. Considering that wild rats rarely compete for access to females [1,37], there is not much reason to expect dominance to have much influence on fertility. The lack of competition is probably related to the fact that both male and female rats may copulate simultaneously with several partners [5,6]. This would also make the notion that prolonged copulatory behavior contributes to mate guarding rather unlikely. In the present study, no relationship between the duration of male copulatory behavior and fertility was found.

In conclusion, present data show that non-sexual, social behavior among rats is unrelated to reproductive success. Nor is the sexual behavior of mounting related to fertility.

The number of ejaculations, and its prerequisite, intromissions, performed are related to reproductive success but they are far from being the only or even the main determining factor of fertility. In fact, the proportion of the variance in fertility that can be attributed to the number of ejaculations or intromissions is around 15 %. Other factors, for example the position of the seminal plug [38] or the quality of sperm [10,11] might be far more important than characteristics of copulatory behavior. The rather modest role of copulatory behavior as a determinant of reproductive success may suggest that selective pressure on that behavior has not been particularly intense. That could perhaps explain the large variation in copulatory behavior between mammalian species. In rabbits, for example, the entire copulatory act consists of one single vaginal penetration, always accompanied by an ejaculation, which lasts for a few seconds at most [39]. To the contrary, males of the species *Antechinus agilis*, a marsupial, spend more than five h mounted on the female. They display a large number of periods with pelvic thrusting before achieving ejaculation [40]. Despite the considerable time and effort involved in each ejaculation, multipaternity is very common among *Antechinus* females [41], just as it is in rabbits [42]. This example illustrates that widely different patterns of mammalian copulatory behavior may have quite similar biological consequences.

It must also be pointed out that most of the data reported in the present contribution could not have been obtained in a short test of copulatory behavior in an opposite-sex pair of rats. The seminatural environment seems to provide a substantial amount of relevant information not available in simpler test procedures. It is even possible that the data reported herein have considerable external validity.

Acknowledgements

Financial support was received from the Faculty of Health Sciences, University of Tromsø. Knut Olsen and Truls Traasdahl assembled the seminatural environment. Carina Sørensen, Katrine Harjo and Nina Løvhaug provided excellent care of the rats.

References

1. Calhoun JB (1962) The ecology and sociology of the Norway rat. Washington,D.C.: US Governnment Printing Office. 288 p.

2. Robitaille JA, Bouvet J (1976) Field observations on the social behavior of the Norway rat, *Rattus norvegicus* (Berkenhout). Biol Behav 1: 289-308.

3. Barnett SA (1975) The rat: a study in behavior. Chicago: University of Chicago Press. 318 p.

4. Telle HJ (1966) Beitrag zur kenntnis der verhaltensweise von ratten, vergleichend dargestellt bei *Rattus norvegicus* und *Rattus rattus*. Z Angew Zool 53: 129-196.

5. Chu X, Ågmo A (2014) Sociosexual behaviors in cycling, intact female rats (*Rattus norvegicus*) housed in a seminatural environment. Behaviour 151: 1143-1184.

6. Chu X, Ågmo A (in press) Sociosexual behaviors of male rats in a seminatural environment. J Comp Psychol

7. Dewsbury DA, Hartung TG (1980) Copulatory-behavior and differential reproduction of laboratory rats in a 2-male, 1-female competitive situation. Anim Behav 28: 95-102.

8. King C, Winstanley T, Innes J, Gleeson D (2014) Multiple paternity and differential male breeding success in wild ship rats (*Rattus rattus*). New Zeal J Ecol 38: 76-85.

9. Miller SD, Russell JC, MacInnes HE, Abdelkrim J, Fewster RM (2010) Multiple paternity in wild populations of invasive *Rattus* species. New Zeal J Ecol 34: 360-363.

10. Birkhead TR, Pizzari T (2002) Postcopulatory sexual selection. Nat Rev Genet 3: 262-273.

11. Dewsbury DA (1982) Dominance rank, copulatory behavior, and differential reproduction. Q Rev Biol 57: 135-159.

12. Lanier DL, Estep DQ, Dewsbury DA (1979) Role of prolonged copulatory-behavior in facilitating reproductive success in a competitive mating situation in laboratory rats. J Comp Physiol Psych 93: 781-792.

13. Andersson M, Simmons LW (2006) Sexual selection and mate choice. Trends Ecol Evol 21: 296-302.

14. Drickamer LC, Gowaty PA, Holmes CM (2000) Free female mate choice in house mice affects reproductive success and offspring viability and performance. Anim Behav 59: 371-378.

15. Blanchard DC, Fukunaga-Stinson C, Takahashi LK, Flannelly KJ, Blanchard RJ (1984) Dominance and aggression in social groups of male and female rats. Behav Processes 9: 31-48.

16. Carr WJ, Kimmel KR, Anthony SL, Schlocker DE (1982) Female rats prefer to mate with dominant rather than subordinate males. B Psychonomic Soc 20: 89-91.

17. Thor DH, Carr WJ (1979) Sex and aggression: competitive mating strategy in the male rat. Behav Neural Biol 26: 261-265.

18. Coria-Avila GA, Pfaus JG, Hernandez ME, Manzo J, Pacheco P (2004) Timing between ejaculations changes paternity success. Physiol Behav 80: 733-737.

19. Lovell JL, Diehl A, Joyce E, Cohn J, Lopez J, et al. (2007) "Some guys have all the luck": mate preference influences paced-mating behavior in female rats. Physiol Behav 90: 537-544.

20. Shimmin GA, Sofronidis G, Bowden DK, Templesmith PD (1995) DNA-fingerprinting to determine paternity in laboratory rat sperm competition experiments. Electrophoresis 16: 1627-1632.

21. Spinka M (1990) The effect of time of day on sperm competition and male reproductive success in laboratory rats. Physiol Behav 47: 483-488.

22. Zewail-Foote M, Diehl A, Benson A, Lee KH, Guarraci FA (2009) Reproductive success and mate choice in Long-Evans rats. Physiol Behav 96: 98-103.

23. Lehner PN (1998) Handbook of ethological methods. Cambridge U.K.; New York: Cambridge University Press. 672 p.

24. Beach FA, Jordan L (1956) Sexual exhaustion and recovery in the male rat. Q J Exp Psychol 8: 121-133.

25. Rodríguez-Manzo G, Fernández-Guasti A (1994) Reversal of sexual exhaustion by serotonergic and noradrenergic agents. Behav Brain Res 62: 127-134.

26. Lucio RA, Rodriguez-Piedracruz V, Tlachi-López JL, García-Lorenzana M, Fernández-Guasti A (2014) Copulation without seminal expulsion: the consequence of sexual satiation and the Coolidge effect. Andrology 2: 450-457.

27. Austin D, Dewsbury DA (1986) Reproductive capacity of male laboratory rats. Physiol Behav 37: 627-632.

28. Tlachi-López JL, Eguibar JR, Fernández-Guasti A, Lucio RA (2012) Copulation and ejaculation in male rats under sexual satiety and the Coolidge effect. Physiol Behav 106: 626-630.

29. Toner JP, Adler NT (1985) Potency of rat ejaculations varies with their order and with male age. Physiol Behav 35: 113-115.

30. Gowaty PA, Drickamer LC, Schmid-Holmes S (2003) Male house mice produce fewer offspring with lower viability and poorer performance when mated with females they do not prefer. Anim Behav 65: 95-103.

31. Costello AK, Pultorak JD, Meikle DB (2009) Do male house mice (*Mus musculus*) discriminate between females that differ in nutritional status? Behav Processes 82: 119-125.

32. Taylor GT, Weiss J (1987) Behavior and fecundity of female rats mated with preferred or non-preferred males. Anim Behav 35: 115-121.

33. Winland C, Bolton JL, Ford B, Jampana S, Tinker J, et al. (2012) "Nice guys finish last": influence of mate choice on reproductive success in Long-Evans rats. Physiol Behav 105: 868-876.

34. Moore CL, Wong L (1992) Copulatory-Behavior, Reproduction, and Sperm Competition in 2 Strains of Male-Rats. Physiol Behav 51: 569-573.

35. Ehman KD, Scott ME (2004) Microsatellite analysis reveals that female mice are indiscriminate when choosing infected or dominant males in an arena setting. Parasitology 129: 723-731.

36. Hinson ER, Hannah MF, Norris DE, Glass GE, Klein SL (2006) Social status does not predict responses to Seoul virus infection or reproductive success among male Norway rats. Brain Behav Immun 20: 182-190.

37. Barnett SA (1958) An analysis of social behavior in wild rats. Proc. Zool. Soc. Lond. 130: 107-152.

38. Matthews MK, Adler NT (1978) Systematic interrelationship of mating, vaginal plug position, and sperm transport in the rat. Physiol Behav 20: 303-309.

39. Contreras JL, Beyer C (1979) A polygraphic analysis of mounting and ejaculation in the New Zealand white rabbit. Physiol Behav 23: 939-943.

40. Shimmin GA, Taggart DA, Temple-Smith PD (2002) Mating behavior in the agile antechinus *Antechinus agilis* (*Marsupialia: Dasyuridae*). J Zool 258: 39-48.

41. Shimmin GA, Taggart DA, Temple-Smith PD (2000) Sperm competition and genetic diversity in the agile antechinus (*Dasyuridae: Antechinus agilis*). J Zool 252: 343-350.

42. Falcón W, Goldberg CS, Waits LP, Estes-Zumpf WA, Rachlow JL (2011) First record of multiple paternity in the pygmy rabbit (*Brachylagus idahoensis*): evidence from analysis of 16 microsatellite loci. West N Am Naturalist 71: 271-275.

Figure Legends

Fig. 1. Behavioral comparison between Offspring pairs (N = 14) and Non-offspring pairs (N = 20). (A) male behaviors (B) female behaviors. Data are mean + SEM. **Mann-Whitney *U* test, $p < 0.01$.

Fig. 2. Behavioral difference between Multiple paternity (N = 8) and Single paternity (N = 6). (A) male behavior (B) female behavior. Data are mean + SEM. *Mann-Whitney *U* test $p < 0.05$, **$p < 0.01$.

Fig. 3. Behavioral difference between Dominant males (N = 4) and Subordinate males (N = 4). (A) copulatory acts (B) fertility and (c) female preference. Data are mean + SEM.

Tables

Table 1. Description of registered behaviors [5,6].

Male and female behavior	**Data collected as**	**Behavior description**
Sniffing	Duration	The rat places its snout close to any body part, except the anogenital region, of another rat while its whiskers move briskly.
Anogenital sniffing	Duration	The rat sniffs, occasionally grooms and licks, another rats' anogenital region.
Pursuit	Duration	The rat runs closely behind another rat.
Fight	Duration	Two rats form a tight ball, rolling around while biting.
Nose-off	Duration	Facing another rat either standing on 4 legs or while rearing; it includes boxing and teeth showing.
Flight	Number	The rat runs away from another rat after an agonistic interaction.
Male copulatory behavior		
Mount	Number	The rat stands on its hind legs and places its forepaws on another rat's rump from behind and displays pelvic thrusting.
Intromission	Number	Mount associated with penile insertion. The mount is ended by a backward thrust and is followed by genital grooming.
Ejaculation	Number	Penile insertion lasts longer than at intromission and is associated with rhythmic abdominal contractions. Dismount is slow and associated with an open arm posture.
Female behavior		
Paracopulatory behavior	Duration	Approach to a male followed by runaway, often associated with hops, darts, ear wiggling.
Lordosis	Number	Female stands immobile with the back arched downward and the rump pushed upward while the tail is deflected to the side.
Rejection	Number	The rat kicks, bites or turns around against its suitor.

Table 2. Correlation coefficient between male fertility and within-pair interactions in all pairs with completely known paternity (N = 34).

	Number of pups	Proportion of pups
Proportion of pups	0.992**	-
Male behaviors		
Mount	-0.013	-0.017
Intromission	0.385*	0.396*
Ejaculation	0.423*	0.435*
Ejaculation ratio	0.458**	0.433[a]*
Intromission ratio	0.420*	0.442[a]**
Pursuit of female	0.037	0.036
Sniffing female	-0.108	-0.094
Anogenital sniffing female	-0.061	-0.047
Number of bouts	-0.015	-0.015
Sum of bout duration	-0.059	-0.039
Female behaviors		
Lordosis	0.055	0.063
Rejection	-0.122	-0.108
Flight	0.071	0.055
Paracopulatory behavior	-0.073	-0.056
Sniffing male	0	0.029
Anogenital sniffing male	0.018	0.030
Nose - off of male	0.029	0.034

*Spearman's rho $p < 0.05$, **$p < 0.01$; [a]Pearson correlation * $p < 0.05$, **$p < 0.01$.

Table 3. Correlation coefficient between male fertility and total male sociosexual behaviors with estrus females during the observation period (N = 13).

	Number of pups	Proportion of pups
Proportion of pups	0.969**	-
Mount	-0.137	-0.237
Intromission	-0.207	-0.315
Ejaculation	-0.151	-0.259
Intromission ratio	0.310	0.327[a]
Copulatory rate	0.123	0.213
Postejaculation interval	-0.268	-0.271
Pursuit of females	-0.084	-0.22
Sniffing females	-0.315	-0.377
Anogenital sniffing females	0.407	0.209
Number of bout	-0.112	-0.209
Sum of duration of bouts	-0.318	-0.407

**Spearman's rho $p < 0.01$, [a]Pearson correlation.

Fig. 1

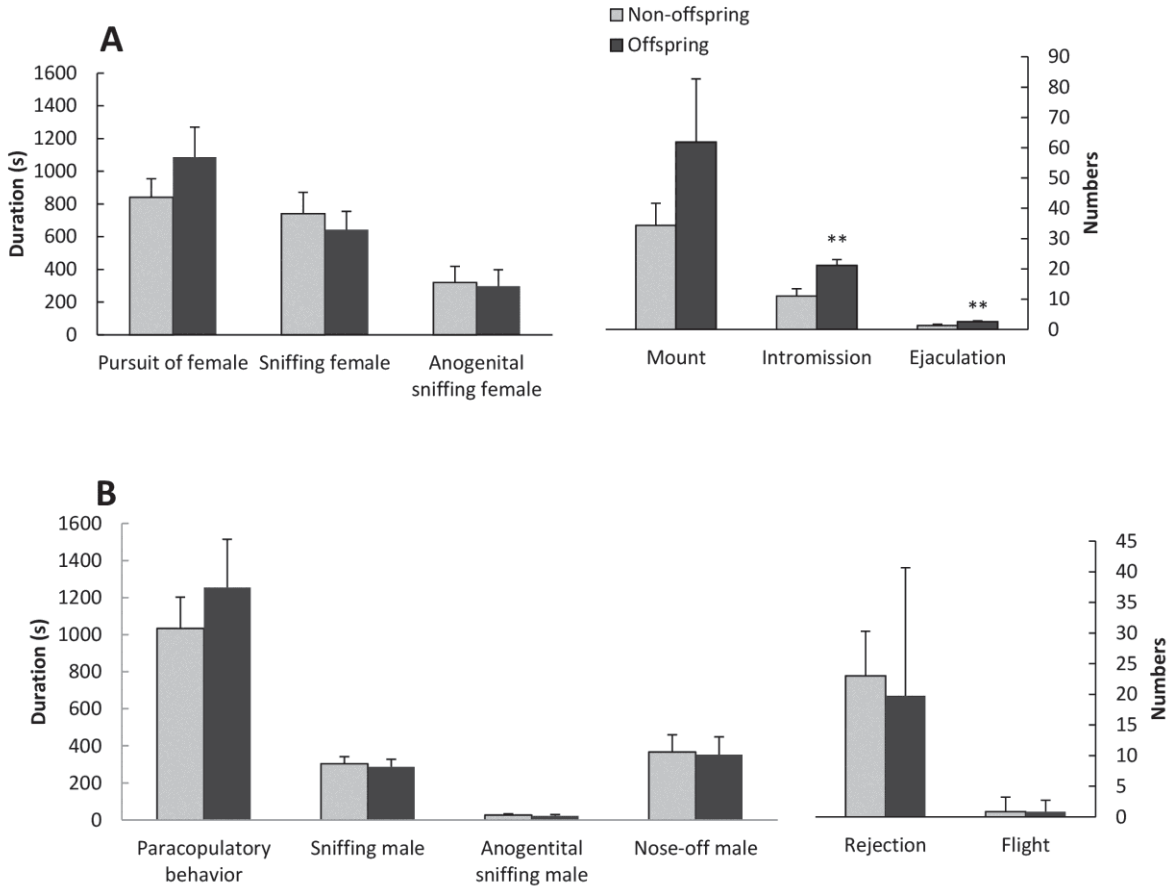

A
Non-offspring
Offspring
Duration (s)
Numbers
1600
1400
1200
1000
800
600
400
200
0
90
80
70
60
50
40
30
20
10
0
**
**
Pursuit of female
Sniffing female
Anogenital sniffing female
Mount
Intromission
Ejaculation
B
Duration (s)
Numbers
1600
1400
1200
1000
800
600
400
200
0
45
40
35
30
25
20
15
10
5
0
Paracopulatory behavior
Sniffing male
Anogentital sniffing male
Nose-off male
Rejection
Flight

Fig. 2

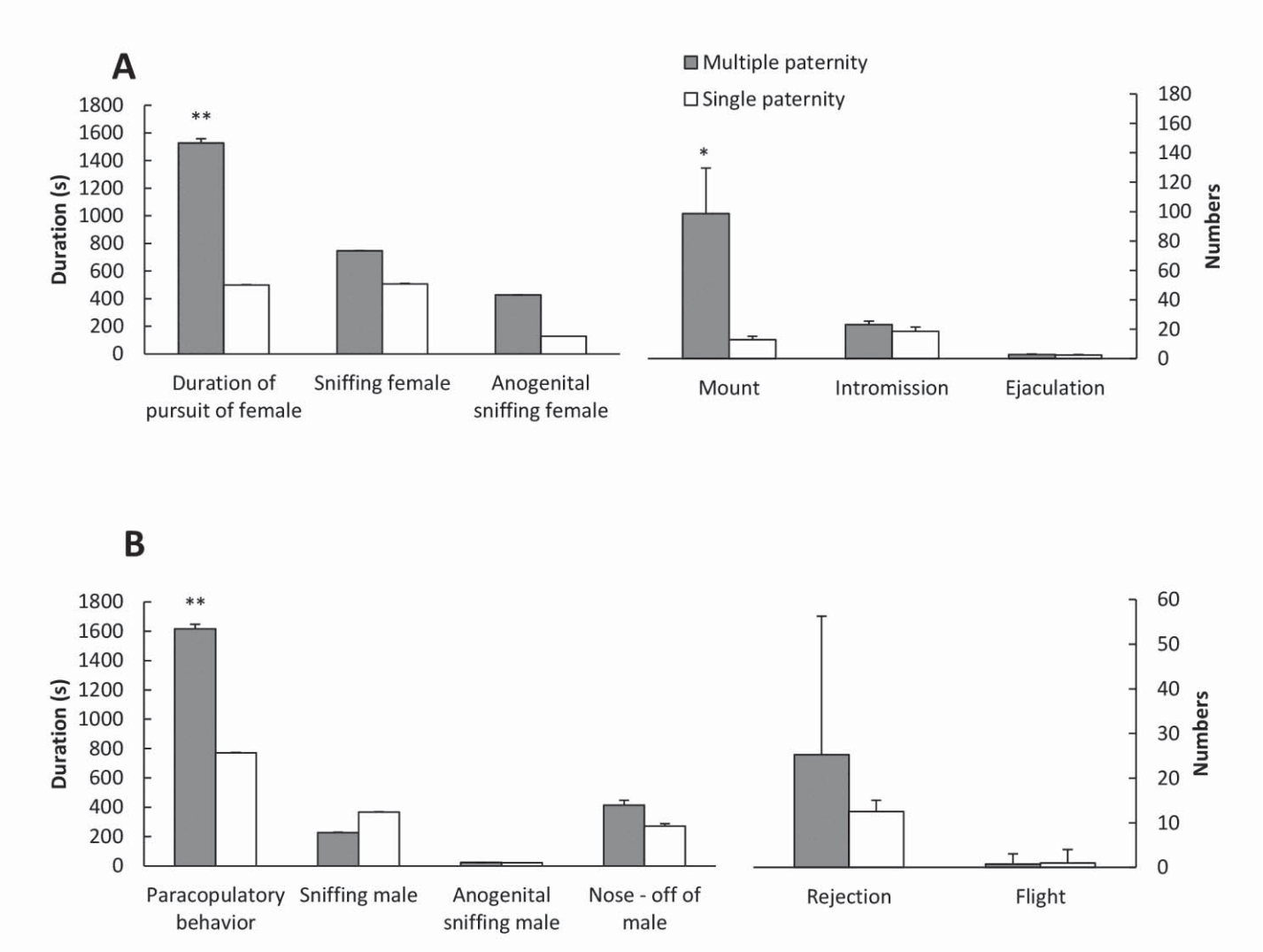
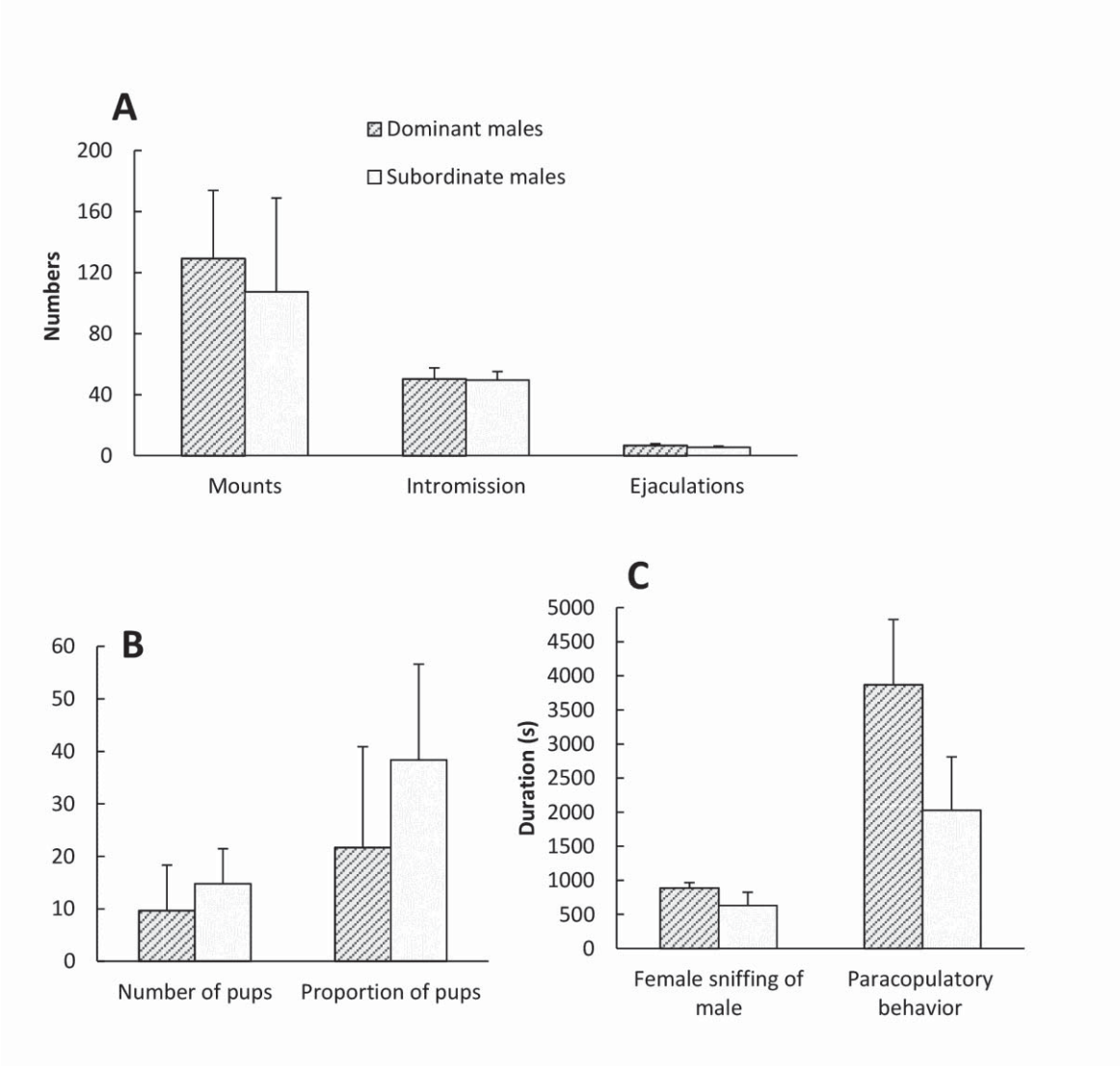

A
Multiple paternity
Single paternity
Duration (s)
Numbers
1800
1600
1400
1200
1000
800
600
400
200
0
180
160
140
120
100
80
60
40
20
0
**
*
Duration of pursuit of female
Sniffing female
Anogenital sniffing female
Mount
Intromission
Ejaculation
B
Duration (s)
Numbers
1800
1600
1400
1200
1000
800
600
400
200
0
60
50
40
30
20
10
0
**
Paracopulatory behavior
Sniffing male
Anogenital sniffing male
Nose - off of male
Rejection
Flight

Fig. 3


A
Dominant males
Subordinate males
Numbers
200
160
120
80
40
0
Mounts
Intromission
Ejaculations
B
60
50
40
30
20
10
0
Number of pups
Proportion of pups
C
Duration (s)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Female sniffing of male
Paracopulatory behavior