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Stud males and dud males: intra-uterine position effects on the reproductive success of male gerbils

MERTICE M. CLARK, LEANNE TUCKER & BENNETT G. GALEF, JR
Department of Psychology, McMaster University, Hamilton, Ontario L8S 4K1, Canada

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Abstract. Sexually mature male Mongolian gerbils, *Meriones unguiculatus*, from known intra-uterine positions were each paired with a succession of nulliparous females. Those males that had matured in intra-uterine positions adjacent to two males (2M males) sired an average of 28% more young than did those males that had matured in intra-uterine positions between two females (2F males). In a second experiment, female gerbils chose between pairs consisting of one adult 2F male gerbil and one adult 2M male gerbil. When in induced oestrus (but not when in other hormonal states), the females spent significantly more time near 2M than near 2F males. Female gerbils in oestrus thus tended to affiliate with the more reproductively capable male in their vicinity.

Natural selection acts only on heritable traits (Lewontin 1978; Enders 1986). However, environmentally induced phenotypic variability correlated with reproductive success is also important to the student of evolutionary process. Such non-heritable variance in fitness can reduce the probability that those individuals carrying more fit genes will, in fact, achieve greater reproductive success than will those individuals carrying less fit genes. Consequently, non-heritable variance in individual fitness will affect the rate at which evolution can proceed. Given the potential importance to evolutionary process of non-heritable variation in reproductive success, there has been surprisingly little study of its sources in animal populations.

Results of previous studies in our laboratory have revealed dramatic effects of intra-uterine position, a non-heritable characteristic, on the reproductive life histories of female Mongolian gerbils, *Meriones unguiculatus*. Ninety-five per cent of female gerbils that mature in intra-uterine positions between two female fetuses (2F females) exhibit vaginal introitus before reaching 25 days of age; only 41% of female gerbils from intra-uterine positions between two male fetuses (2M females) exhibit such early maturation (Clark et al. 1986; Clark & Galef 1989). Female gerbils exhibiting vaginal introitus before reaching 25 days of age have a lifetime fecundity more than twice as great as that of their late-maturing sisters (Clark et al. 1986). It is easily calculated that, on average, 2F female gerbils wean 1·4 times as many young as do 2M female gerbils.

It has also been found that the intra-uterine positions that male rodents occupy as fetuses affects their exposure to androgens during the perinatal period (vom Saal 1989; Clark et al. 1990, 1991). There is evidence consistent with the view that adult male gerbils that matured in 2M intra-uterine positions have higher plasma levels of testosterone than adult male gerbils that matured in 2F intra-uterine positions. Adult 2M male gerbils (1) scent mark more frequently, (2) have greater anogenital distances and (3) have larger ventral glands than 2F male gerbils (Clark et al. 1990); each of these characteristics is correlated with high circulating levels of testosterone (Thiessen et al. 1968; Blum & Thiessen 1971). Furthermore, recent radioimmunoassays of plasma testosterone concentrations in adult male gerbils have consistently revealed significantly higher circulating levels of testosterone in 2M than in 2F males (Clark et al., unpublished data).

The intra-uterine positions that male rodent fetuses occupy can also affect the copulatory pattern that they exhibit when adult. Both 2M male gerbils and 2M male house mice, *Mus domesticus*, mount oestrus females more rapidly and ejaculate after fewer intromissions and with shorter mean latencies than do 2F conspecifics (vom Saal et al. 1983; vom Saal 1989; Clark et al. 1990).

In sum, behavioural and morphological correlates of intra-uterine position are well established in

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male rodents. However, there is no evidence that this phenotypic variability affects reproductive success of males, as it does reproductive success of females. Our experiments were undertaken to determine whether the intra-uterine positions occupied by male Mongolian gerbils affected their ability to attract (experiment 2) and impregnate (experiment 1) females.

EXPERIMENT 1

Our aim in experiment 1 was to examine the probability of male Mongolian gerbils from different intra-uterine positions impregnating females to which they had unlimited access.

Methods

Subjects

Subjects were 280 female and 35 male Mongolian gerbils born in the vivarium of the McMaster University Psychology Department to breeding stock acquired from Tumblebrook Farm (Brookfield, Massachusetts). All 280 females were delivered vaginally and were reared by their natural dams. Each male was taken from a litter that was: (1) Caesarean delivered on day 24 of gestation, (2) toe-clipped at birth for individual identification and (3) foster reared by a dam that had given birth naturally on the day of Caesarean delivery of the litter she was to rear. Details of methods of Caesarean delivery and foster rearing are given in Clark & Galef (1988).

When a litter reached 35 days of age, it was weaned and its members were placed in same-sex groups of two to four siblings in polypropylene cages measuring 35 × 30 × 15 cm with lids of 1·3-cm hardware cloth and floors covered with a thin layer of wood-chip bedding. When each male reached 53 days of age, it was placed alone in a cage until initiation of the experiment, when it was 60 days old.

Female subjects were weaned at 35 days of age and were maintained in same-sex sibling groups of three or four until they were 60–70 days of age, when each was paired with a male subject.

All subjects were fed Purina Rodent Laboratory Chow 5001 and water ad libitum and were maintained on a 12:12 h light:dark cycle in a single colony room controlled for temperature and humidity.

Classification of the intra-uterine position of males

As each pup in a Caesarean-delivered litter was removed from its dam's uterus, we determined the sex of that pup on the basis of its anogenital distance (Raible & Gorzalka 1987; Clark & Galef 1990) and recorded its position in its dam's uterus (Clark & Galef 1988). We classified those male fetuses located between two female fetuses as 2F males and those male fetuses located between two male fetuses as 2M males. Twenty 2F males and 15 2M males from 35 different litters served as subjects. It should be noted that 2M males are, by definition, also OF males, as defined in Clark et al. (1990).

Breeding of males

Each male gerbil was tested for reproductive capacity by pairing it with a succession of eight, nulliparous female gerbils 60 to 70 days of age. To begin a breeding test, a single female gerbil was introduced into a male's cage. The male was removed from each pair's cage 20 days later and the female was left undisturbed until she either gave birth or until 25 days had passed without her giving birth. After removal from the cage containing its mate, each male was placed in a new cage and was left undisturbed for 6 to 10 days before another female was introduced into his cage.

We checked each female's cage twice/day (0900 and 1700 hours) and recorded both the date on which parturition occurred and the number of pups in each litter that females delivered.

Results

Males that had matured in 2M intra-uterine positions sired more offspring than did males that had matured in 2F intra-uterine positions (Fig. 1). Analysis of the causes of these differences in reproductive success indicated, first, that a 2M male was significantly more likely to impregnate a female with which it was paired for 20 days than was a 2F male (Table I). As a result of 271 pairings of females with males, we observed 36 failures of females to reproduce: 30 following pairing with 2F males and six following pairing with 2M males.

Second, those females successfully impregnated by 2F males gave birth to smaller litters than did those females impregnated by 2M males, although this result was not statistically significant ($P<0.08$; Table I).

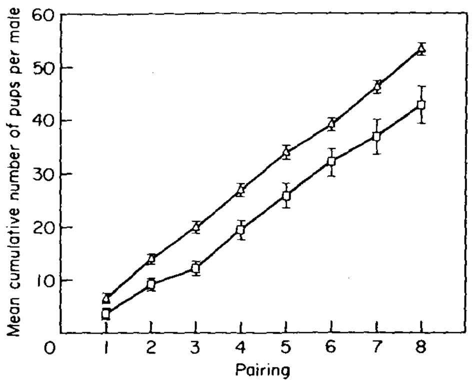

Figure 1. Mean ($\pm$ SE) cumulative number of pups sired by male Mongolian gerbils from 2M ($\triangle$) and 2F ($\square$) intra-uterine positions as a result of eight successive pairings with nulliparous female gerbils.

Mean time from pairing to parturition did not vary as a function of the intra-uterine positions that males had occupied. Presumably, 2F males that did impregnate females did so as rapidly as did 2M males.

If we eliminated from consideration results of the first pairing of each male with a female (a pairing that occurred when males were relatively immature), 2M males were still more likely to impregnate females than were 2F males (Student's $t = 1{\cdot}97$, $df = 31$, $P < 0{\cdot}05$) and 2M males clearly sired more young per litter than did 2F males (Student's $t = 2{\cdot}27$, $df = 31$, $P < 0{\cdot}03$).

Discussion

Taken together the results of experiment 1 suggest that some of the variability in reproductive success of adult male Mongolian gerbils can be understood in terms of differences in the uterine environments in which they matured. Male gerbils from 2M intra-uterine positions were more reproductively successful than were male gerbils from 2F intra-uterine positions.

EXPERIMENT 2

In discussions of criteria used by female vertebrates in mate selection, attention has focused on selection by females of (1) males of high genetic quality, whose gametes might enhance the probability of projection of a female's own genetic complement into future generations (Partridge 1980), and (2) males controlling access to physical resources (territories, feeding sites, nuptial gifts, etc.) that might enhance a female's reproductive success (Searcy 1982). There are, however, considerations other than the genetic quality of males and males' control of physical resources of reproductive value to females that might be expected to influence a female's choice of mate. For example, in those species in which males can monopolize sexual access to females, a female should be reluctant to pair with a male that is relatively unlikely to impregnate her, even if the male's reduced potency is not heritable.

In experiment 1, we found that potency of adult, male Mongolian gerbils was correlated with the intra-uterine positions that they occupied as fetuses. One might therefore predict, on functional grounds, that if female gerbils could discriminate between males from different intra-uterine positions, they would choose to mate with more potent 2M males, rather than with less potent 2F males, and thus maximize their chances of impregnation during mating.

As mentioned in the Introduction, results of previous studies indicate that adult male gerbils from 2M intra-uterine positions, have higher levels of circulating testosterone than do adult male gerbils from 2F intra-uterine locations. Differences in circulating levels of androgens are known to provide a sensory basis for discrimination among males by females in rodent species other than gerbils (Taylor et al. 1982). It is, therefore, not unreasonable to suppose that female gerbils might be able to discriminate between adult males of their species that had matured in 2M and 2F intra-uterine positions using cues correlated with differences in their circulating levels of androgens.

Our aim in experiment 2 was to determine whether female gerbils in oestrus (and therefore susceptible to impregnation) would exhibit a tendency to affiliate with relatively potent, 2M male gerbils rather than with less potent, 2F male gerbils.

Methods

Subjects

Subjects were 30 female Mongolian gerbils, 90–110 days old, that had been ovariectomized when 60 days of age. Ten Caesarean-delivered, male Mongolian gerbils, 180–200 days old, each from a separate litter served as stimulus animals. As fetuses, five of these stimulus males had matured in

Table I. Mean (±SE) measures of reproductive success of male Mongolian gerbils as a function of their intra-uterine positions

	2M (N=15)	2F (N=18*)	t	df	P†
Pups/male/pairing	6·8±0·1	5·3±0·4	2·90	31	0·007
Females bearing young (%)	95·0±2·0	79·8±5·8	2·28	31	0·028
Pups/male/pairing‡	7·0±0·1	6·6±0·2	1·78	30	NS
Days: pairing to parturition‡	29·9±0·4	30·0±0·4	0·03	30	NS

*Two 2F males died in the course of the experiment. One impregnated four of five females, the other two of three females with which it was paired before it died.
†All Ps for two-tailed Student's t-test.
‡Refers only to those matings producing young.

intra-uterine positions between two females (2F males) and five had matured in intra-uterine positions between two males (2M males).

Each of the 30 female gerbils participating in this experiment were taken from one of 30 litters born in the vivarium of the McMaster University Department of Psychology to stock acquired from Tumblebrook Farm (Brookfield, Massachusetts). Each litter was weaned when 25 days of age and its members were then maintained in groups of three or four same-sex siblings for 35 days. When each litter was 60 days old, we randomly selected a single female from that litter to serve as a subject in the experiment.

Both male and female subjects were housed individually in polypropylene shoe-box cages, measuring 15 × 30 × 15 cm, from the time that they were 60 days of age until the start of the experiment and all were maintained as in experiment 1.

Apparatus

Each female subject was tested for her preference between males in a rectangular cage measuring 75 × 21·3 × 20·6 cm, constructed of black Perspex. The cages were divided into three compartments by two fixed 1·3-cm hardware-cloth partitions (21·3 × 20·6 cm) and associated removable opaque partitions constructed of black Perspex. During testing, a female was placed in the central compartment, which measured 30 × 21·3 × 20·6 cm, of the apparatus and one stimulus male was placed in each of its two smaller side compartments, which measured 22·5 × 21·3 × 20·6 cm.

Procedure

We conducted testing of each female in three phases: pre-injection, injection and post-injection. During each phase, each female was tested once a week for 5 weeks for her preference between the members of a different pair of stimulus males. Across the 15 trials of the experiment, each subject female chose between the members of 15 different pairs of stimulus males.

To begin each trial in each phase of the experiment, we placed a female gerbil in the central compartment of the apparatus and introduced a 2M male into one side compartment and a 2F male into the other. After a 5-min period of habituation, we removed the opaque partitions concealing the side compartments from the female in the central compartment. During the next 15 min, an observer, unaware of the intra-uterine position in which each stimulus male had matured, recorded the amount of time that a female spent in the half of the central compartment closer to each stimulus male. Following each 15-min test, the apparatus was cleaned with a 70% alcohol solution.

Pre-injection phase

Over a period of 5 weeks, we tested each of the 30 female subjects for preference between five different pairs of 2M and 2F males during five, 15-min test trials.

Injection phase

Upon completion of the 5-week pre-injection phase of the experiment, we assigned each female subject to one of three experimental treatments (N= 10/treatment). Assignment of females to treatment conditions was based on their behaviour during the pre-injection phase of the experiment. Each subject female was assigned a score equal to her mean percentage preference for 2M males during the 5 days of the pre-injection phase. Assignment of subject females to treatment conditions was counterbalanced so as to equate as closely as possible the mean percentage preference for 2M males during the pre-injection phase of subjects assigned to each treatment condition in the injection phase.

We began testing of subjects in the injection phase of the experiment 1 week after completion of the pre-injection phase. Subjects in the testosterone group (group T) received injections of 300 mg of testosterone propionate both 4 days and 1 day before each day of testing (Thiessen et al. 1968). Subjects in the oestradiol group (group E) were brought into oestrus by subcutaneous injections of 10 mg of oestradiol benzoate both 2 days and 1 day before each test day and injection of 500 mg of progesterone 5–6 h before each test day (Raible & Gorzalka 1987). Subjects in the control group (group C) were injected with 0·1 ml of safflower oil when subjects in group E were injected with either oestradiol benzoate or progesterone.

During the 5-week injection phase, each female subject received five 15-min tests of preference between five different pairs of 2M and 2F males.

Post-injection phase

Following completion of the injection phase of the experiment, each subject female was left undisturbed for 30 days to permit dissipation of any hormones administered during the injection phase. At the end of the 30-day interval following completion of the injection phase, 5 weeks of tests, similar to those completed during the pre-injection and injection phases of the experiment were carried out. Once again, each female chose between five new pairs of 2M and 2F stimulus males.

Testing males

Seven days after completion of the post-injection phase of the experiment, we measured frequency of scent marking by each of the 10 stimulus males. On each of 4 consecutive days, each stimulus male was placed in a test arena with an opaque, white Perspex floor (92 × 92 cm) and shellacked wooden walls 62 cm high. The arena floor was divided into 16 squares (23 × 23 cm) by lines painted on the floor surface and a black 0·5 cm high Perspex peg (1 × 2 cm) was attached to the floor at each of the nine points of intersection of the painted lines.

A male gerbil was placed in the enclosure and an observer, unaware of the intra-uterine position that that subject had occupied as a fetus, recorded the number of times the subject scent marked during the 10 min following its introduction into the apparatus. Scent marking was defined as an active lowering of the belly and dragging of the ventral gland pad across either a peg or the floor of the arena (Thiessen et al. 1968).

Following examination of scent marking, we anesthetized each male subject. The experimenter, unaware of the intra-uterine positions in which a subject had developed, then determined the maximum length and width of its ventral scent gland.

Results

During the pre-injection phase, females to be assigned to the three experimental treatments differed neither from one another ($F_{2,27}=0{\cdot}26$, NS) nor from chance ($\bar{X}\pm\text{SE}=49{\cdot}6\pm0{\cdot}8\%$) in the proportion of 15-min test periods they spent near 2M males (Fig. 2).

During the injection phase of the experiment, the tendency of females to remain near 2M males was clearly affected by females' hormonal states ($F_{2,27}=12{\cdot}27$, $P<0{\cdot}001$). Comparison of the mean percentage of test periods that females spent near 2M males during pre-injection and injection stages of the experiment revealed that (1) 8 of the 10 females in group E increased the amount of time that they affiliated with 2M males (sign test, $x=2$, $P<0{\cdot}05$), (2) all 10 females in group T decreased the percentage of time they spent near 2M males (sign test, $x=0$, $P<0{\cdot}001$), and (3) females in group C did not show a change in tendency to affiliate with 2M males (sign test, $x=5$, NS).

During the post-injection phase of the experiment, females in the three groups did not differ either from one another ($F_{2,27}=0{\cdot}001$, $P>0{\cdot}10$) or from chance ($\bar{X}\pm\text{SE}=48{\cdot}7\pm1{\cdot}3\%$) in their tendency to affiliate with 2M males.

Testing of males revealed, consistent with our previous findings (Clark et al. 1990), that 2M males scent marked significantly more frequently than did 2F males ($\bar{X}\pm\text{SE}$ for 2M males, $23{\cdot}1\pm5{\cdot}5$ marks/day; for 2F males, $13{\cdot}0\pm2{\cdot}8$; Mann–Whitney U-test, $U=3$, $P=0{\cdot}045$) and that 2M males had significantly larger ventral scent glands than did 2F males ($\bar{X}\pm\text{SE}$ for 2M males, $1{\cdot}73\pm0{\cdot}12\ \text{cm}^2$; for 2F males, $1{\cdot}39\pm0{\cdot}08\ \text{cm}^2$; Mann–Whitney U-test, $U=3$, $P=0{\cdot}045$).

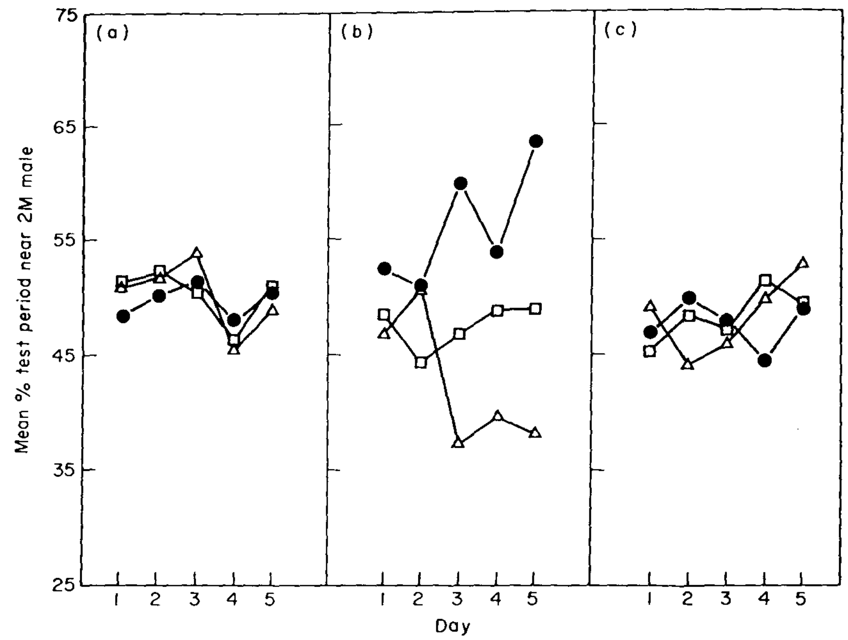

Figure 2. Mean percentage of 15-min test trials that female subjects in group E (●), group T (△) and group C (□) spent on the side of the central compartment closer to 2M males. (a) Pre-injection, (b) injection and (c) post-injection phases of experiment 2.

Pearson's rank-order correlations between the mean preference of females in oestrus for individual males and individual males' scent-marking scores and ventral-gland sizes (Table II) revealed that females in oestrus preferred those males with larger scent glands and higher frequencies of scent marking, while male choice by females injected with testosterone correlated with neither the size of males' scent glands nor their frequencies of scent marking.

Table II. Rank-order correlations between (1) 2M males' attractiveness to females during the injection phase of the experiment (Pref), (2) scent-marking frequencies (SMF) and (3) ventral gland sizes (VGS) of 2M males

	Pref	SMF	VGS
Pref	—	0·98*	0·90*
SMF	0·10	—	0·90*
VGS	0·40	0·90*	—

Entries above the diagonal are for subject females in group E. Entries below the diagonal for subject females in group T.
*$P<0{\cdot}05$.

Discussion

The results of experiment 2 (1) show that female gerbils are able to discriminate between adult males from different fetal intra-uterine positions, (2) suggest that such discriminations are based on differences in circulating levels of androgens to be found in adult males from different intra-uterine positions and (3) show that a female's tendency to affiliate with males from different intra-uterine positions depends on her hormonal status. In particular, our results show that females in oestrus tend to prefer 2M males to 2F males and that this preference is steroid-hormone specific in that females injected with testosterone exhibited preferences opposite those exhibited by females injected with oestradiol.

The results of experiment 2 also reveal a second way in which males from 2F intra-uterine positions are at a reproductive disadvantage relative to males from 2M intra-uterine positions. 2F males are not only less potent than 2M males, they are also less attractive to females in reproductive condition.

It is, perhaps, relevant to note that Agren et al. (1989) have observed that, when in oestrus, female Mongolian gerbils in natural habitat sometimes leave the territory of the male with which they are paired to copulate with a male from an adjacent territory. Our data are consistent with the notion that this unexpected pattern of behaviour may be an adaptive response by female gerbils living in the territories of males with relatively low circulating levels of androgen and, therefore, of relatively low potency. By temporarily abandoning a consort with low reproductive potential for the company of a more virile neighbour, a female gerbil in oestrus may increase either her probability of becoming pregnant or the size of the litter she will deliver.

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