Unconfounded Evidence of Sex-Biased, Postnatal Maternal Effort by Mongolian Gerbil Dams

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In previous studies of effects of offspring sex on postnatal maternal effort there has often been unintentional confounding of the sex ratio of the pups that a dam reared with the sex ratio of the litter that she gestated. By controlling the sex ratio of litters that female subjects gestated, we showed that (1) the probability of dams retrieving pups was influenced by the sex ratio of the litters dams gestated, not by the sex of litters that dams reared and (2) both frequency of nursing and duration of vaginal closure were influenced by the sex of litters that dams reared, not by the sex ratios of litters that dams gestated.

In a recently published paper (Clark, Bone, & Galef, 1990), we reported evidence consistent with the hypothesis that the postpartum investment which female Mongolian gerbils (*Meriones unguiculatus*) make in sons is greater than the postpartum investment that they make in daughters. In comparison with gerbil dams rearing 4 daughters, dams rearing 4 sons: (1) delivered significantly fewer young as a consequence of postpartum mating, (2) exhibited significantly longer interlitter intervals, (3) exhibited significantly longer periods of postpartum vaginal closure (vaginal closure prevents intromission and vaginal reopening is correlated with cessation of milk transfer from dams to young) and (4) were more attentive mothers (sons were both more likely than daughters to be gathered together in the nest and to be found attached to their dam's nipples.

As indicated in a previous discussion of these findings (Clark et al., 1990), there is a methodological problem in all published studies of sex bias in maternal

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Received for publication 12 April 1991
Revised for publication 15 July 1991
Accepted by Wiley 22 September 1991

Developmental Psychobiology 24(8):539–546 (1992)

CCC 0012-1630/92/080539–08$04.00

investment that is the result of partial (or complete) self-assignment of dams to rearing conditions. In general, dams giving birth to female-biased litters are more likely than dams giving birth to male-biased litters to be assigned by an experimenter to rear daughters. Conversely, dams giving birth to male-biased litters are more likely than dams giving birth to female-biased litters to be assigned by an experimenter to rear sons. To take an extreme case, it is impossible to assign a dam that delivers only daughters to rear her sons. In less extreme cases, dams that give birth to male- or female-biased litters inadvertently tend to be assigned to rear young of the more common sex. For example, in our study of sex bias in maternal investment by Mongolian gerbils (Clark et al., 1990), post-hoc analysis revealed that dams assigned to rear sons had given birth to litters containing (Mean ± 1 *SE*) 64.2 ± 3.3% males, while dams assigned to rear daughters had given birth to litters containing 29.1 ± 3.9% males.

Gerbil dams that give birth to male-biased litters tend to give birth to small litters, to have long interbirth intervals, and to be attentive mothers, while dams that give birth to female-biased litters are more likely to give birth to large litters, to have relatively short interbirth intervals, and to be relatively inattentive mothers (Clark, Spencer & Galef, 1986). Consequently, the observation by Clark et al. (1990) that, relative to gerbils rearing daughters, gerbils rearing sons exhibited longer interbirth intervals, reduced future fecundity, and enhanced maternal care may have had nothing to do with differences in the relative postnatal maternal effort made by dams rearing sons and daughters. Those females that tended toward low fecundity, long interlitter intervals, and enhanced maternal care may have also been those females that were more likely to give birth to male-biased litters and, because of the sex ratios of their litters at birth, tended to be assigned to rear sons rather than to rear daughters.

There are two ways to avoid such nonrandom assignment of dams to rearing conditions in studies of sex bias in maternal investment (Clark et al., 1990): First, one can use as subjects only those dams giving birth to litters composed of equal numbers of male and female young and then randomly assigned half such dams to rear their sons and half to rear their daughters. Alternatively, one can use as subjects any dam giving birth, but can randomly assign her to rear a fixed number of foster pups of either one sex or the other.

Reported here are the results of two partial repetitions of Clark et al. (1990), unconfounded by bias in the sex ratios of litters born to dams assigned to rear sons or daughters. One repetition used as subjects dams that gave birth to exactly 4 sons and 4 daughters that were then randomly assigned to rear either their sons or their daughters. In the second repetition, dams that gave birth either to male- or female-biased litters were assigned to rear either 4 male or 4 female foster young.

In all the studies described here, males were removed from their mates' cages when females were obviously pregnant, so no impregnation occured during postpartum estrus. Consequently, we could not examine directly effects of the sex of pups reared by a dam on her subsequent reproductive behavior. We could, however, examine effects of the sex of pups in the litter that a dam reared and effects of the sex of pups a female gestated on her overt maternal effort.

Methods

Subjects

Subjects were 150 primiparous female Mongolian gerbils, descendants of breeding stock acquired from Tumblebrook Farm, Brookfield, MA. Subjects were selected from among a much larger sample of gerbil dams and their litters born in the vivarium of the McMaster University Department of Psychology over a period of 4 yr. Thirty of the dams selected for the experiment gave birth to exactly 4 male and 4 female pups. The remaining 120 dams each gave birth to at least 4 male or at least 4 female pups and to 3 or fewer pups of the other sex.

The breeding colony was maintained on ad libitum Purina Rodent Laboratory Chow 5001 and water in a single temperature- and humidity-controlled colony room on a 12:12 hr light/dark cycle (light onset at 0500 hr).

Procedure

Breeding

When 60 days of age, each potential female subject was placed with an adult male in a polypropylene shoe-box cage (35 × 30 × 15 cm) with a hardware cloth lid and floor covered in wood-chip bedding.

Each breeding pair was examined daily, and when a female was conspicuously pregnant (i.e., late in the second or early in the third week of her pregnancy), her mate was removed from the cage. On the day that a female delivered her litter, the sex of each pup was determined by inspection of anogenital distance (Clark & Galef, 1990) and if the composition of a dam's litter was appropriate, assigned her to 1 of 3 studies described below.

Study 1: Dams either delivering male-biased litters and rearing sons or delivering female-biased liters and rearing daughters (40 litters). On the day of birth of a litter containing: (1) at least 4 male pups and fewer female than male pups (a male-biased litter, $N = 20$) or (2) at least 4 female pups and fewer male than female pups (a female-biased litter, $N = 20$), the litter was culled to 4 pups of the majority sex. On the day of their birth, the 40 male-biased litters used in the experiment contained (Mean ± 1 *SE*) 67.6 ± 2.5% male pups, while the 40 female-biased litters used contained 32.9 ± 2.1% male pups.

Study 2: Dams delivering exactly 4 male and 4 female pups and rearing 4 sons or 4 daughters (30 litters). On the day of birth of a litter containing exactly 4 male and 4 female pups, the litter was culled either to 4 male pups ($N = 15$ litters) or 4 female pups ($N = 15$ litters). Each dam was then left to rear her remaining offspring.

Study 3: Dams delivering either male- or female-biased litters and rearing 4 male or 4 female foster pups (80 litters). On the day of birth of a male-biased ($N = 40$) or female-biased ($N = 40$) litter, as defined in the description of Study 1, each dam was given 4 foster pups to rear. These pups were members of a litter born on the same day that a dam delivered her own litter.

Twenty of the 40 females giving birth to male-biased litters and 20 of the 40 females giving birth to female-biased litters were each given 4 male foster pups to

Table 1
Mean Number of Pups and Sex Ratios at Birth of Litters Born to Dams Assigned to Each of the Four Conditions in Study 3

Dams rearing:	Litter at birth: Male-biased		Female-biased	
	Males	Females	Males	Females
Litter size	7.5*	7.6	7.6	7.5
% males	66.6(2.4)**	67.7(2.4)	33.1(2.4)	34.2(1.9)

*N = 20 litters/cell
** = Mean (± 1 SE)

rear. Similarly, 20 of the 40 dams giving birth to male-biased and 20 of the 40 females giving birth to female-biased litters were each given 4 female foster pups to rear. Table 1 shows the actual mean litter size at birth and mean sex ratio at birth of litters born to dams assigned to each of the four foster-rearing conditions.

Testing

Three indices of dams' maternal effort were employed: a measure of nursing frequency, a measure of probability of retrieving young, and a correlate of renewed sexual activity (Clark et al., 1990), the number of days from parturition until a dam's vagina reopened.

Nursing

An observer, unaware of the gender of the pups individual dams were rearing, examined each dam's cage twice daily (1000 & 1400 hr) from the day of birth of her litter (Day 1) to the day of its weaning (Day 35). The observer recorded whether each dam was in a nursing posture over her pups and whether one or more pups were attached to her nipples.

Retrieval

Each dam's tendency to retrieve pups was assessed on Day 10 postpartum. An experimenter, again unaware of the gender of the pups a female was rearing, lifted a dam off her nest, removed two pups, lowered the dam back onto her nest, and placed the two removed pups in the corner of the cage farthest from the nest. Five min later, the experimenter determined whether the female had retrieved one or both of her displaced pups.

Vaginal reopening

Each dam was examined daily from Day 20 to Day 35 postpartum to determine the date on which her vagina reopened. During the period of vaginal closure, intromission is not possible.

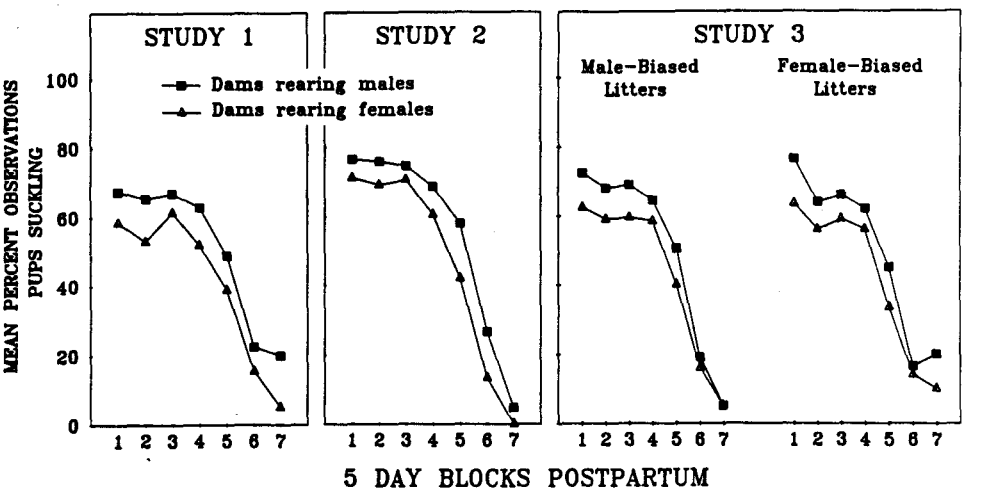

Fig. 1. Percentage of daily observations when females were nursing pups.

Results and Discussion

Study 1

Because some of our dependent variables were measured differently in the present experiment than in the experiments reported in Clark et al. (1990), the first data analyses were undertaken to determine whether the current methods would replicate our previous report that the sex ratios of litters gestated and reared by gerbil dams affected both their maternal behavior and the duration of their vaginal closure after parturition (Clark et al., 1990). The behavior of the 20 dams that gave birth to male-biased litters and reared 4 of their own sons was compared with the behavior of the 20 dams that gave birth to female-biased litters and reared 4 of their own daughters. These 40 dams, assigned to Study 1, reared young under conditions most similar to those of dams observed by Clark et al. (1990), i.e., they gave birth to sex-biased litters, reared 4 of their own young, either male or female, and the gender of the young that each dam reared was congruent with the sex bias of the litter delivered.

As can be seen in Figures 1, 2, and 3, we found in Study 1 that, in comparison with dams rearing daughters, dams rearing sons: (1) were more likely to be observed with their young attached to their nipples (Fig. 1; $F(1, 38) = 5.15, p < .03$), (2) were more likely to retrieve one or both of their displaced young (Fig. 2; Fisher Exact Probability Test, $p = .03$) and (3) exhibited delayed vaginal reopening (Fig. 3; $t(38) = 2.83, p < .01$). These differences between dams rearing four sons and dams rearing four daughters exactly paralleled those reported in Clark et. al. (1990). We conclude: (1) that previously reported differences in maternal effort of gerbil dams are robust to variations in experimental methods and (2) that the present methods, though less time consuming than those used in Clark et al. (1990), are sufficiently sensitive to detect effects of sex of young on dams' maternal efforts.

Study 2

Study 2 permitted examination of the maternal effort of dams that gave birth to litters of fixed size (8 pups) and fixed sex ratio at birth (1:1) and then reared

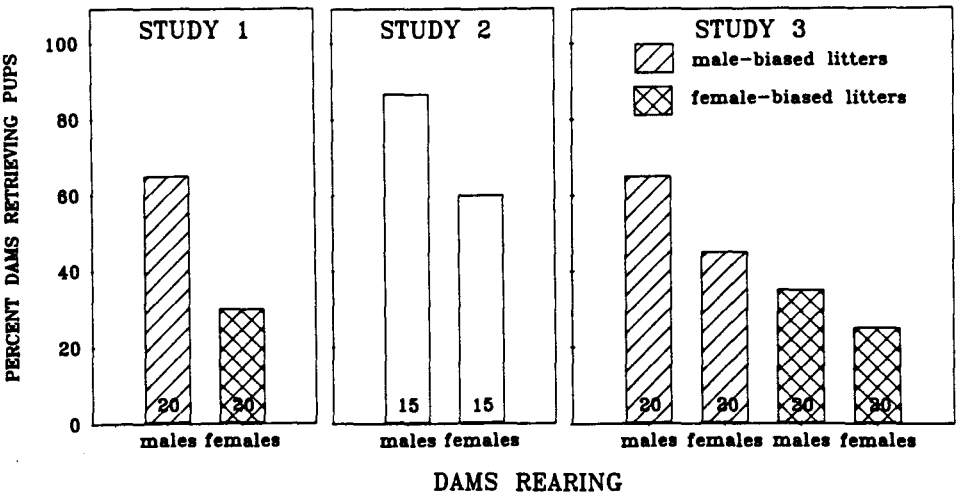

Fig. 2. Mean percentage of dams retrieving one or both pups during testing on Day 10 postpartum.

either 4 of their own sons or 4 of their own daughters. Any differences in the maternal effort of such dams must be attributed to the sex of the pups that they were assigned to rear, not to the size or sex ratio of the litter that they had gestated, as size and sex ratio of gestated litters were equated for all subjects.

As can be seen in Figures 1, 2, and 3 respectively, in comparison to dams in Study 2 rearing their own daughters, dams in Study 2 rearing their own sons: (1) were more likely to be found nursing their young, $F(1,28) = 6.13, p < .02$, (2) were slightly, but not significantly, more likely to retrieve pups (Fisher's Exact Probability = ns), and (3) exhibited significantly longer delays to vaginal re-

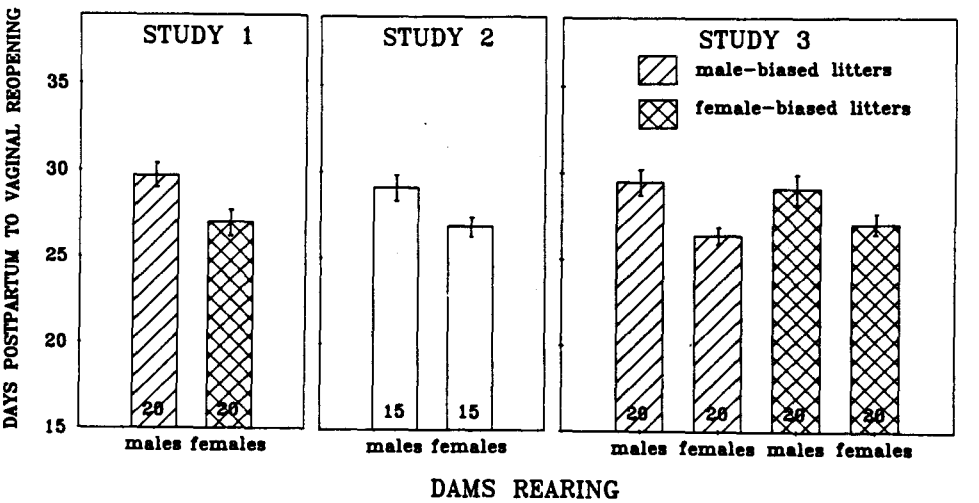

Fig. 3. Mean days postpartum to vaginal reopening.

opening, $t(28) = 2.45, p < .02$. Thus, 2 of our 3 indices of a dam's maternal effort were affected significantly be the sex of her nurslings independent of the sex of the fetuses that she carried through gestation.

Study 3

The results of Study 3 pointed to the same conclusions as did the results of Study 2. As can be seen in Figure 1: (1) Dams giving birth to both male- and female-biased litters and rearing male foster pups were more likely to be seen nursing their young than were comparable dams rearing female foster pups, $F(1,76) = 8.66, p < .005$ and (2) the sex ratio of the litter a female gestated did not significantly affect the probability of nursing her foster litter, $F(1,76) = .40, p =$ ns.

On the other hand, as can be seen in Figure 2, the probability that a dam would retrieve displaced pups was affected by the sex ratio of the litter she gestated, not the sex of the pups she reared: (1) Dams that had given birth to male-biased litters were more likely to retrieve pups than were females giving birth to female-biased litters, $X^2 = 5.10$, $df = 1$, $p < .05$. and (2) dams rearing male pups were as likely as were dams rearing female pups to retrieve during testing (Fisher Exact Probability Test = ns).

As can be seen in Figure 3, both dams giving birth to male-biased litters and dams giving birth to female-biased litters exhibited longer delays to vaginal opening when rearing male pups than when rearing female pups, $F(1,76) = 14.30, p < .001$. Sex bias of gestated litters had no effect on delay to vaginal opening, $F(1,76) = .03, p =$ ns. Thus, like probability of nursing, delay to vaginal opening was affected by the sex of the pups that a dam was rearing, not the sex of the pups she carried to parturition.

Summary

Taken together, the results of the present experiment indicate that in studies of maternal behavior and maternal investment, there is need to control for biases introduced by possible nonrandom assignment of dams to experimental conditions. Although nursing and vaginal reopening were not influenced by the sex ratio of the litter that a dam gestated, retrieval was so influenced. Consequently, in studies in which sex ratio of a dam's litter at birth affected her probability of assignment to rear sons or daughters, her retrieval of pups would appear to be influenced by the sex of the pups she was rearing, when retrieval was in fact influenced by the sex ratio of the litter gestated. Explicit control of potential bias in assignment of dams to treatment conditions is necessary to avoid errors in interpretation of results of experiments on maternal behavior.

Although the present data provide no evidence concerning the way in which the sex ratio of the litter a female was gestating or nursing might have affected her maternal behavior, it is worth noting that we have previously reported significant positive correlations between both the number and the percentage of males in litters that female gerbils gestate and their plasma testosterone levels (Clark, Crews, & Galef, 1991). Bridges and Russell (1981) have provided evidence that during pregnancy, testosterone acts together with progesterone to facilitate later expression of female rats' postpartum retrieval of their young. The finding in the

present experiment that gerbil dams gestating male-biased litters (and, therefore, experiencing relatively high levels of testosterone during pregnancy) were more likely to retrieve pups than were dams gestating female-biased litters (and, therefore, experiencing relatively low levels of testosterone during pregnancy) is consistent with Bridges and Russell's (1981) evidence that exposure to testosterone during pregnancy facilitates postnatal expression of retrieval behavior.

Notes

This research was supported by a grant from the Natural Sciences and Engineering Research Council of Canada to M. M. C. We thank Elaine Whiskin for technical assistance and Martin Daly for a thoughtful critique of an earlier draft of the manuscript.

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