

Facultative sex-ratio adjustment in Norway rats: litters born asynchronously are female biased

Mark S. Blumberg^{1,2}, Julie A. Mennella^{1,3}, Howard Moltz, and Martha K. McClintock¹

¹ Committee on Biopsychology, Department of Psychology, The University of Chicago, 5730 Woodlawn Avenue, Chicago, IL 60637, USA

² Department of Psychology, University of Iowa, Iowa City, IA 52242, USA

³ Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104, USA

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Summary. Previously we reported that inter-litter competition reduces the survival of pups born to pairs of female rats living and breeding in the same nesting environment. Inter-litter competition occurred when females gave birth asynchronously; specifically, when a female gave birth in the presence of 15 to 28-day-old pups, her newborn pups were likely to die as a result of nest intrusion by the older pups. In contrast, inter-litter competition occurred rarely when the two females gave birth synchronously. Because theories of facultative sex ratio adjustment predict that mothers giving birth in unfavorable circumstances should bias their offspring towards the more viable or less expensive sex, we predicted that litters born asynchronously would be female biased. Conversely, we also predicted that females giving birth under favorable conditions, i.e., synchronously, would bias their litters toward males. We found a female bias in asynchronous litters, but did not find a male bias in synchronous litters. Moreover, in contrast to other reports in the literature, the female bias in asynchronous litters was achieved without a reduction in litter size. Based on correlational data, we suggest several mechanisms that could produce this female bias: conditions at fertilization and implantation, time since the male last mated and number of pups suckling concurrently during gestation.

Introduction

Female Norway rats (*Rattus norvegicus*) typically conceive and rear young within a social group in the presence of other breeding females (Calhoun 1962; McClintock 1981, 1983, 1984; Telle 1966; Steiniger 1950). Thus, one might expect the timing of reproductive events within a rat colony to have significant consequences for the reproductive success of each female, as it does in other communally breeding species (e.g., lions *Panthera leo*,

Bertram 1975). Indeed, inter-litter competition reduces the survival of offspring born to female rats living and breeding in the same nesting environment (Mennella et al. 1990); when two females give birth asynchronously (i.e., 15–28 days apart), the younger litter usually dies soon after birth as a result of nest intrusion by the older pups (such a litter is hereafter designated a high-risk litter). In contrast, when two females give birth synchronously (i.e., 0–14 days apart), both litters have a high chance of survival; the survival rate of these litters is similar to that of litters born when no other pups were present (both synchronous and only litters are hereafter designated low-risk litters). In addition to enhanced survival, low-risk pups weigh more at weaning than do high-risk pups, perhaps because the former are often nursed by their own mother as well as by the mother of the other litter.

According to sex ratio theory, the circumstances of the mother affect her “decision” to invest in one sex more than the other; this differential investment can be manifested as a biased sex ratio at birth (Clutton-Brock 1991). In general, parents are expected to invest more heavily in the sex that provides a greater return in parental fitness (Silk 1983). Under specific conditions, Trivers and Willard (1973) hypothesized that a mother in good condition should produce more males than females and, conversely, a mother in poor condition should produce more females than males. Both aspects of the Trivers-Willard hypothesis have been supported, although rarely in the same species, leading to qualifications of the original formulation (see critical literature reviews by Hrdy 1987 and Clutton-Brock 1991; see also Altmann et al. 1988; Clark 1978; Clutton-Brock et al. 1984; Labov et al. 1986; McClure 1981; Meikle et al. 1984; Pratt et al. 1988; Silk 1983).

In her critique of the Trivers-Willard hypothesis, Myers (1978) argued that we must be careful to distinguish between sex ratio adjustments that involve differential mortality of young (and thus reduced total reproductive output) and sex ratio adjustments that occur without a reduction in litter size. For example, if a female

experiencing poor conditions produces a predominantly female litter as a result of a higher mortality rate of males in response to prenatal stress, it is not clear whether one can consider this an adaptive adjustment of sex ratio because the total reproductive output of the mother is reduced. On the other hand, if a female responds to poor conditions by adjusting the primary sex ratio toward the less energetically expensive sex without reducing the total number of young, then such an adjustment would be a better candidate for an adaptive response.

Therefore, based on Myers' argument, a female rat in poor condition that adjusts the sex ratio of her offspring facultatively should produce more females than males without reducing litter size. One would expect an over-production of females because, from an energetic standpoint, female rats are less expensive to raise than are males. For example, female rat pups weigh less at birth (Hedricks and McClintock 1990; King 1915) as well as at weaning (Mennella et al. 1990). Female pups also receive less maternal attention, insofar as mothers lick the ano-genital region of female pups less than they do male pups (Moore and Morelli 1979).

Thus, in rats, whether one considers the future reproductive potential of offspring (Trivers-Willard) or the energetic cost of raising offspring (Myers), the mother of an asynchronous high-risk litter should bias her offspring toward females. Moreover, if such a female bias occurs without a decrease in litter size, then such a sex biasing would be a stronger candidate for a facultative adjustment than if male mortality simply increased. Conversely, one might also expect the mother of a synchronous low-risk litter to bias her offspring toward males. We tested these predictions by examining previously-collected data (Mennella et al. 1990).

Method

Animals. The animals and environmental conditions were identical to those described elsewhere (Mennella et al. 1990). Wistar rats were born in the laboratory and reared in mixed-sex litters. Following weaning at 30 days of age, they were housed with animals of the same sex and age in groups of six to eight. A lighting schedule of 12L:12D was in effect throughout the study. The ambient temperature was $22 \pm 2^\circ \text{C}$ with a relative humidity of 60–90%.

At approximately 90 days of age (range: 77–109 days old), 18 virgin female rats were chosen as experimental subjects. From this pool, nine pairs of females of equal age were constituted. The two females in each pair had been housed together following weaning. One female of each pair was marked with methyl violet dye so that the two females could be distinguished throughout the study.

Environment. The nesting environment was constructed to provide some of the physical complexity typical of a rat burrow (Calhoun 1962; McClintock 1984). Plywood (2.54 cm) was joined to enclose an area $120 \times 120 \times 60$ cm and covered with 0.64 cm hardware cloth. A partition with two open doorways divided the area in half. One side of the environment was an open area containing food and water as well as a platform of wire mesh, a cylindrical tunnel of wire mesh and a large brick; the other side contained two adjacent nest boxes constructed of red Plexiglas. The floor of each environment was covered with pine chips for sanitation and shredded newspaper for nesting material.

Procedure. Four to six days after a given pair of females was placed in the environment, a sexually-experienced male was introduced; these males had not copulated for at least 14 days. Each triad of two females and one male remained in its assigned environment for a period potentially sufficient to allow each female to give birth to and wean three litters.

Dates of fertilization were determined by looking for sperm in the vaginal smear of each female at the beginning of the study and within 1 day following the birth of each litter, at which time the female experiences a postpartum estrus. In six cases, postpartum mating did not result in the birth of a litter within 40 days (Vorherr and Vorherr 1984), perhaps as a result of suppression of postpartum fertility (Gudermuth et al. 1984). Because of this, and because the next litter was necessarily conceived in a cycling estrus, we inferred the date of fertilization for these litters by counting back 22 days from the day of parturition (the length of a typical pregnancy conceived at a cycling estrus; Vorherr and Vorherr 1984; Smart and Dobbing 1971).

Each triad was observed at least once daily for the duration of the study. During these observation periods we noted when each female first appeared pregnant. We also recorded the date of birth of a litter, the number of pups alive and the number of pups nursed by each female on each postpartum day. During these daily observations, only the pups were handled. If a litter was born within 1 week of another, the pups in one of the litters were toe-clipped for identification. Within 1 day of parturition, we recorded the number of males and females in each litter and, if the litter survived to weaning (i.e., to approximately 30 days of age), we recorded the number of males and females at that time also.

Statistical analyses. Sex biasing of litters was determined and tested in two ways. First, the secondary sex ratio, defined as (number of males born)/(number of pups born), was calculated for each litter. This sex ratio is a proportion and was therefore arcsine root transformed to meet the requirements of normative statistics (arcsine $\sqrt{\text{SR}}$ where SR is the sex ratio; Pollard 1977). Parametric tests were then applied to test for significant bias in sex ratio as well as to test for differences between high-risk and low-risk groups. Second, we tested for bias among the litters of a given group by determining the number of males and females born in each litter of that condition and performing a Wilcoxon matched-pairs signed-ranks test. A reduction in the variability of the sex ratio was tested using a χ^2 test (for a detailed description of this method and its derivation, see the appendix to Huck et al. 1990). Finding less variance than expected by chance would be evidence of selection for a facultative bias in sex ratio and could result from a number of mechanisms (Williams 1979; James 1975). All statistical comparisons were performed using Statview II on the Macintosh II computer. Summary statistics are expressed as mean \pm SEM.

Results

Sex-ratio bias

A total of 42 litters were born, all of which were divided into two groups on the basis of their risk of high mortality from inter-litter competition. High-risk litters were defined as those litters born in the presence of pups who were 15–28 days of age. Of the 42 litters 13 were designated high-risk. Of these, 92% were female biased and 8% were male biased at birth ($P < 0.005$; Wilcoxon; see Fig. 1). In addition, the mean sex ratio of 0.391 ± 0.029 was significantly different from 0.500 ($t = -3.70$; $df = 12$; $P < 0.003$). The designation "high-risk" was justified since only 28% of the pups in these litters survived to weaning (Mennella et al. 1990).

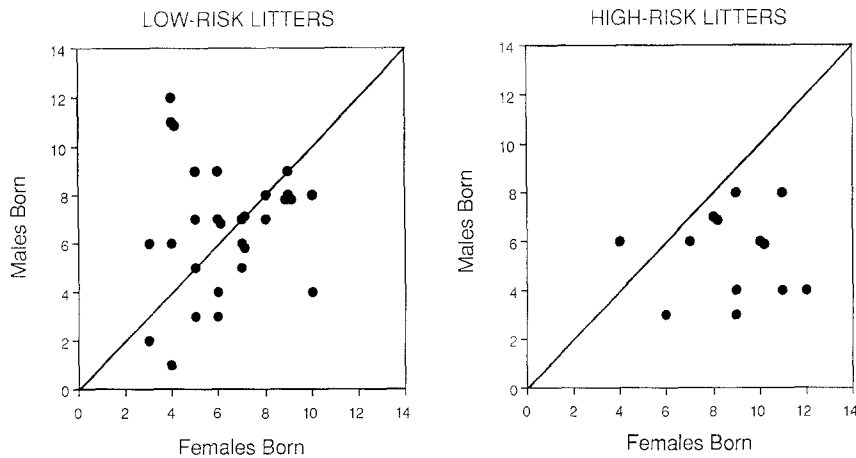


Fig. 1. Number of females born versus number of males born in low-risk and high-risk litters. The diagonal is the isometric line indicating a 50:50 sex ratio

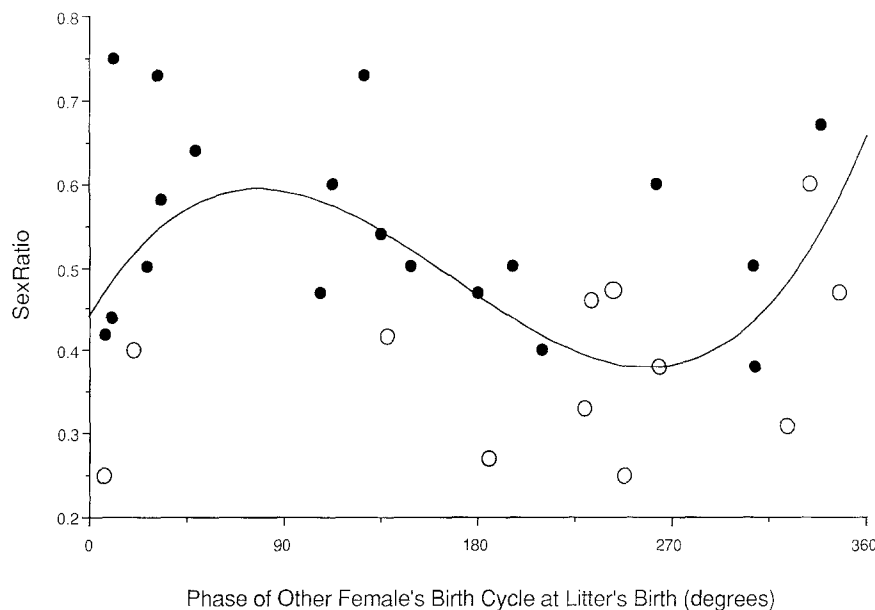


Fig. 2. Sex ratio as a function of degree of birth cycle synchrony. Degree of synchrony is measured for all litters in this study as the percentage of the other female's birth cycle (inter-birth interval) that had elapsed when the litter was born. Filled circles are low-risk litters, open circles are high-risk litters. We fit the data using a 3rd-order polynomial equation

Low-risk litters were defined as those litters born either in the presence of young pups (0–14 days of age) or no pups. Of the 29 low-risk litters, 48.3% were female biased, 34.5% were male biased, and 17.2% were unbiased (NS; Wilcoxon; see Fig. 1). In addition, the mean sex ratio of 0.501 ± 0.024 was not significantly different from 0.500 ($t=0.08$; $df=28$; NS), but was significantly different from the mean sex ratio of high-risk litters ($t=2.72$; $df=40$; $P \leq 0.01$). The designation “low-risk” was justified since 80% of the pups in these litters survived to weaning (Mennella et al. 1990).

Thus, the data support the hypothesis that mothers giving birth under unfavorable conditions will give birth to more females than males; however, the data do not support the converse, that is, that mothers giving birth under favorable conditions will give birth to more males than females.

Sex ratio bias and birth cycle synchrony

Offspring sex ratio was significantly correlated with the degree of birth cycle synchrony (3rd-order polynomial

correlation; $r=0.49$; $n=31$; $P \leq 0.06$; 1st-, 2nd-, and 3rd-order terms, all P 's ≤ 0.05). Figure 2 depicts this sinusoidal relationship. This figure was derived by determining, for each litter, the percentage of the other female's inter-birth interval (IBI) that had elapsed when the litter was born. For example, if a litter was born at a time when 50% of the other female's IBI had elapsed, then the two females were asynchronous; their birth cycles were 180° out of phase. Similarly, if a litter was born at a time when 0% or 100% of the other female's IBI had elapsed, then the two females were synchronous; their birth cycles were in phase (0°). Thus, Fig. 2 indicates that the greatest amount of female bias occurred when a litter was born after 50–75% of the other female's IBI had elapsed, i.e., when their birth cycles were 180°–270° out of phase.

Effect of reproductive-history variables

Determining the putative mechanisms underlying the female bias of high-risk litters was facilitated by the fact

Table 1. Mean sex ratio, litter size, number of males born, number of females born, and birth number for low-risk and high-risk litters

Group	Sex ratio	Litter size	Males born	Females born	Birth number	CE/PPE
Low-risk (<i>n</i> = 29)	0.501 (0.024)	13.1 (0.7)	6.7 (0.5)	6.3 (0.4)	1.4 (0.1)	19/10
High-risk (<i>n</i> = 13)	0.0391 (0.029)	14.3 (0.8)	5.5 (0.5)	8.8 (0.6)	2.5 (0.1)	4/9
<i>P</i> -value	≤ 0.01	NS	NS	≤ 0.005	≤ 0.0001	≤ 0.05

All values are mean (SEM) and all tests are *t*-tests. Also shown is the number of litters conceived in cycling estrus (CE) and postpartum estrus (PPE) in each group. This difference was tested by χ^2

Table 2. Mean sex ratio, litter size, number of males born, number of females born, birth number, gestation length, and number of pups suckled concurrently for low-risk and high-risk litters conceived in a postpartum estrus

Group	Sex ratio	Litter size	Males born	Females born	Birth number	Gestation length (days)	Number of pups suckled concurrently
Low-risk (<i>n</i> = 10)	0.503 (0.031)	11.6 (1.2)	5.9 (0.7)	5.7 (0.6)	2.1 (0.1)	31.9 (0.6)	13.1 (0.8)
High-risk (<i>n</i> = 9)	0.359 (0.029)	14.8 (1.0)	5.3 (0.6)	9.4 (0.6)	2.4 (0.2)	27.3 (1.2)	5.3 (1.4)
<i>P</i> -value	≤ 0.005	≤ 0.06	NS	≤ 0.001	NS	≤ 0.003	≤ 0.0001

All values are mean (SEM). All tests are *t*-tests

that the animals were breeding ad libitum over as many as three birth cycles, producing variation in reproductive history variables known to affect sex ratio and enabling us to test their effects.

Litter size. The litter sizes of high-risk and low-risk litters were not statistically different (see Table 1). In addition, there was no correlation between litter size and sex ratio ($r=0.18$; $n=42$; NS). This finding differs from other studies of sex ratio in rodents (Pratt et al. 1988; Lane and Hyde 1973) in which decreases in sex ratio were accompanied by decreases in litter size; these decreases in litter size were attributed to differential male mortality *in utero* or selective male infanticide at birth. Table 1 indicates, however, that the female bias of high-risk litters is mostly due to an increase in the number of females born, not a decrease in the number of males born. Furthermore, the fact that the female bias of high-risk litters was not accompanied by decreased litter size makes it highly unlikely that the sex ratio bias was due to differential male mortality after birth. Therefore, the mechanism(s) driving the female biasing of high-risk litters appears to be acting before birth.

Parity and female identity. In addition to litter size, parity has also been shown to affect sex ratio (Hedricks and McClintock 1990; Huck et al. 1988). In fact, high-risk litters were never their mother's first-born ($P \leq 0.0003$; $\chi^2=12.8$) and were significantly more likely to be born to multiparous mothers than were low-risk litters (see Table 1). It is possible, therefore, that the female bias of high-risk litters was due to the greater parity of their mothers. We tested this possibility by performing

analyses of variance on the effect of parity on sex ratio. When the first two births of the females were considered, there was no significant effect of parity on sex ratio ($F_{1,33}=0.217$; NS). Similarly, when three births were considered, there was no significant effect of parity on sex ratio ($F_{2,39}=1.442$; NS). Moreover, there was no significant effect of the individual identity of the mother on the sex ratio of her litter (repeated measures analysis of variance $F_{5,17}=2.24$; NS; parity $F_{5,17}=0.40$; NS) just as there was no effect of individual identity on risk for inter-litter competition (Mennella et al. 1990). We conclude from this that neither parity nor identity of the mother can account for the female bias of high-risk litters.

Estrous condition. Many rodents, including rats, mate either during a cycling estrus or within 1 day after parturition during a postpartum estrus. Because females in this study were allowed to breed continuously through as many as three birth cycles, litters were conceived during both cycling estrus and postpartum estrus. As shown in Table 1, 69% (9/13) of the high-risk litters were conceived during postpartum estrus compared to 34% (10/29) of the low-risk litters. This difference is significant ($\chi^2=4.38$; $P<0.05$). It is possible, therefore, that high-risk litters were biased toward females because they tended to be conceived during a postpartum estrus.

Estrous condition, however, had no effect on sex ratio (main effect of estrous condition: $F_{1,38}=1.34$ NS; main effect of high/low risk: $F_{1,38}=4.35$; $P<0.05$; interaction $F_{1,38}=1.48$; NS). This lack of effect can be illustrated by comparing the sex ratios of high- and low-risk litters conceived during postpartum estrus. Specifically, of the

9 high-risk litters conceived during postpartum estrus, 100% were female biased ($P < 0.01$; Wilcoxon). Moreover, the mean sex ratio of 0.359 ± 0.029 was significantly different from 0.500 ($t = 4.726$; $df = 8$; $P < 0.002$) and tended to be less variable than expected by chance ($\chi^2 = 3.88$; $df = 8$; $P < 0.10$). In contrast, of the 10 low-risk litters conceived during postpartum estrus, 50% were female biased, 40% were male biased and 10% were unbiased (NS; Wilcoxon). Moreover, the mean sex ratio of 0.503 ± 0.031 was not significantly different from 0.500 ($df = 9$; $t = 0.116$) but was significantly different from the mean sex ratio of high-risk postpartum-conceived litters ($t = 3.367$; $df = 17$; $P < 0.005$; Table 2). The sex ratio was significantly less variable than expected by chance ($\chi^2 = 3.26$; $df = 9$; $P < 0.05$).

It is clear that conception during a postpartum estrus is not *sufficient* for causing a female bias. Nonetheless, it is possible that conception during a postpartum estrus is *necessary* for causing a female bias. Because we lacked sufficient data to examine thoroughly the effect of high risk on litters conceived in a cycling estrus (we only observed four such litters and three were female biased), we cannot determine if high-risk litters are female biased regardless of the cycling condition of the mother at conception. Nonetheless, it is possible that the uniqueness of postpartum conditions (viz., hormonal status, behavior, and gestation length; Hedricks and McClintock 1985; Gilbert et al. 1980) plays a role in the sex biasing of high-risk litters. For example, the possible effect of suckling stimulation on the sex ratio of a gestating litter is only relevant in the context of a postpartum-conceived litter. To examine such possible effects, we have restricted the remaining analyses to litters conceived during postpartum estrus. Again, the small number of high-risk litters conceived during cycling estrus in this sample precluded a similar detailed analysis of mechanisms that could produce a sex ratio bias.

Correlates of birth-cycle asynchrony

Restricting analysis to litters conceived during postpartum estrus, we found a tendency for high-risk litters to be larger than low-risk litters (14.8 versus 11.6 pups; $t = 2.017$; $df = 17$; $P < 0.06$; see Table 2). This increased litter size was due solely to an increase in the number of females born in high-risk litters (9.4 versus 5.7 females; $t = 4.126$; $df = 17$; $P < 0.001$), once again suggesting that the female bias of high-risk litters is not due to differential mortality of males *in utero*, but rather is due to increased production of female fetuses.

Because there is a sinusoidal relationship between degree of birth synchrony and sex ratio (Fig. 2), we can assume that the mechanisms that produce a female bias in asynchronous high-risk litters is associated with the asynchrony of the birth cycles. In our search for such a mechanism, we have arrived at four variables that are related to the synchrony-asynchrony continuum and that may have contributed to the female bias of high-risk litters. These four variables are (1) conditions at the time of fertilization, (2) time since the male last mated, (3) conditions at the time of implantation, and (4) number

Table 3. Correlations between sex ratio of postpartum-conceived litters and four reproductive variables associated with birth-cycle asynchrony (Pearson's r ; see text for discussion)

Reproductive variables associated with birth-cycle asynchrony	Sex ratio (Pearson's r)	P -value
At time of fertilization, the number of days before the other mother gave birth	0.65 ($n = 16$)	≤ 0.01
At time of fertilization, the number of days since the male last mated	-0.59 ($n = 19$)	≤ 0.01
At time of implantation, the age of the other mother's pups	0.62 ($n = 15$)	≤ 0.02
Minimum number of pups suckling mother during gestation	0.56 ($n = 19$)	≤ 0.02

of concurrent suckling pups during gestation. Given that all of these variables were associated with birth cycle synchrony, it is not surprising that they also tended to be intercorrelated ($0.29 \leq \text{all } r\text{'s} \leq 0.90$; 5 out of 6 correlations were at least $P \leq 0.10$). Nonetheless, they are physically independent conditions. Therefore, the possible role of each variable in determining sex ratio bias is described in succession (see Table 3).

Conditions at fertilization. For each postpartum-conceived litter at the time of its fertilization, we measured the number of days before the other female gave birth. We found that if the other female was pregnant with an implanted litter (i.e., if she was ≤ 16 days before term; Krehbiel 1941), then the mean sex ratio was 0.364 ± 0.028 ($n = 10$), but if the other female's litter had not implanted (e.g., she was 17–33 days before term), then the mean sex ratio was 0.525 ± 0.044 ($n = 6$). This difference was significant ($t = 3.38$; $df = 14$; $P < 0.005$). Not surprisingly, at fertilization, the sex ratio of a postpartum-conceived litter was positively correlated with the number of days before the other female gave birth ($r = 0.65$, $n = 16$, $P < 0.01$). Thus, some pheromonal or behavioral signal from a female carrying an implanted litter (Gudermuth et al. 1984) could affect the sex ratio of another female with whom she lives by altering the conditions during mating or fertilization [for example, by influencing the time of day that mating takes place (Hedricks and McClintock 1990)]. For a mating female, such a signal would be a good indicator that she is conceiving a litter asynchronously with another female and would be at risk of delivering in the presence of older pups (correlation between gestation stage of the other female at a litter's fertilization and the age of the other pups at its birth = 0.89, $P \leq 0.001$). She could then respond to such a signal with a female-biased litter.

Time since the male last mated. With only one male in the environment with the two females, the rest time for

each male between inseminations varied greatly (range = 0–30 days). There was a negative correlation between the length of this rest time and the sex ratios of the postpartum-conceived litters, with rest periods longer than 10 days associated with female-biased litters ($r = -0.59$, $n = 19$, $P < 0.01$). It is possible, therefore, that sexual inactivity affects the timing of a male's mating behavior, and thereby the sex ratio of the litter (Hedricks and McClintock 1990), or it may affect the proportions of X- and Y-bearing sperm in his ejaculate. On the other hand, this correlation was not significant for litters conceived during cycling estrus ($r = 0.13$, $n = 18$, $P > 0.60$). Thus, if the male's behavior or sperm composition were factors in the female biasing of high-risk litters, they were only factors through an interaction with the postpartum condition.

Conditions at implantation. For each postpartum-conceived litter at the time of its implantation (i.e., 16 days before birth), we determined the age of the other mother's pups (range = 3–27 days of age). We found that the age of these pups was positively correlated with sex ratio at birth, with a female bias associated with the presence of pups less than 14 days old ($r = 0.62$; $n = 15$; $P < 0.02$). Thus, younger suckling pups may produce a signal that differentially affects implantation of male or female fetuses and thus the sex ratio of the litter.

Number of pups suckling concurrently during gestation. Females that delivered an asynchronous high-risk litter were likely to have lost their previous litter. Specifically, of the mothers of the nine high-risk litters conceived in postpartum estrus, 56% had previous litters with survival rates less than 20% and therefore few suckling pups concurrent with their pregnancy. In contrast, all ten of the concurrent suckling litters of low-risk litters had survival rates greater than 70%. This difference in survival rate was significant ($\chi^2 = 8.65$, $P < 0.005$). Thus, females delivering high-risk litters were less likely than females delivering low-risk litters to have nursed pups concurrently during the gestation of their litter. Given that suckling stimulation affects implantation, gestation length, and the hormonal milieu of a litter developing *in utero*, we examined the effect of these variables on the sex ratio of the newborn litter.

The number of young suckling concurrently during the gestation of a litter, and thus the degree of suckling stimulation, was estimated from the number of young weaned in the previous litter. This provided a minimum estimate of concurrent suckling stimulation. Comparing concurrent suckling stimulation between high-risk and low-risk litters, mothers of high-risk litters nursed fewer young than did the mothers of low-risk litters (5.3 versus 13.1 pups; $t = 4.89$; $df = 17$; $P < 0.0001$; Table 2). There was a significant positive correlation between the number of concurrent suckling young (range = 0–18 pups) and the sex ratio of the subsequent litter ($r = 0.56$; $n = 19$; $P < 0.02$), with a female bias associated with suckling fewer than nine pups.

It is well established that suckling stimulation affects the gestation length of postpartum pregnancies (Enz-

mann et al. 1932; Krehbiel 1941; Vorherr and Vorherr 1984; Mantalenakis and Ketchel 1966). As expected, the number of pups suckling concurrently was correlated with the gestation length of the subsequent litters ($r = 0.83$; $n = 19$; $P < 0.0001$). Moreover, the gestation lengths of high-risk litters were significantly shorter than those of low-risk litters (27.3 versus 31.9; $df = 17$; $t = 3.582$, $P < 0.003$; see Table 2). Gestation length, however, was not correlated with sex ratio ($r = 0.33$; $n = 19$; NS).

The correlation of the number of concurrent suckling pups with the sex ratio of the subsequent litter remained significant even when the effect of gestation length was removed through partial correlation ($r = 0.54$; $n = 19$; $P < 0.01$). In addition, the correlation between gestation length and sex ratio remained non-significant when the effect of number of concurrent suckling pups was removed through partial correlation ($r = -0.29$; $n = 19$; NS). It appears, therefore, that if the number of concurrent suckling young is affecting sex ratio, it is doing so independently of its effect on gestation length.

Discussion

These results, showing that high-risk litters are female biased, support one aspect of the Trivers and Willard (1973) hypothesis: females giving birth under unfavorable circumstances bias their litters' sex ratios towards females. High-risk litters are born in unfavorable circumstances because they must compete with older pups and, in addition, they are not nursed communally (Mennella et al. 1990). Our data, however, do not support the other aspect of the Trivers-Willard hypothesis, namely that litters should be male biased when born under favorable circumstances, as has been found in synchronously-breeding lions (Packer and Pusey 1987). Thus, this second aspect may not be valid in rats, or, if it is valid, then the two aspects of the hypothesis may operate independently and may be mediated by different mechanisms.

Trivers and Willard (1973) suggested that a female-biased sex ratio could be accomplished through differential male mortality, resulting in smaller litter sizes. Myers (1978) argued, however, that when such reductions in male births and litter size occur, they are the result of greater prenatal susceptibility of males to stress (e.g., Lane and Hyde 1973; Pratt et al. 1988). Furthermore, she argued that such differential mortality with reduced total reproductive output is of doubtful functional value to the mother, and thus these instances of differential male mortality should not be considered adaptive adjustments of sex ratio. Female biases would be truly adaptive, according to her, only when there is female biasing without decreased litter size. Our finding that high-risk litters were female biased without a decrease in litter size is consistent with Myers's more stringent criterion for an adaptive sex-ratio bias and indicates that the mechanism of the bias involves increased production of females, not increased mortality of males.

We suggested a variety of mechanisms, one or all of which may contribute to the female biasing of post-

partum-conceived litters during asynchronous breeding. All of these conditions, however, were significantly associated with asynchronous breeding and hence tended to be associated with each other as well. Thus, choosing among them must await experimental analysis that enables an examination of their effects independently of one another.

We should comment further, however, on one of the potential mechanisms. It will be recalled that postpartum-conceived litters were female biased when they developed *in utero* with few concurrent suckling pups. This bias contrasts sharply with the sex ratio of litters conceived during a cycling estrus; such litters had a sex ratio near parity and yet developed *in utero* with no concurrent suckling pups. The difference in sex ratio between the two estrous conditions, when the number of concurrent suckling pups is similar, suggests an interesting hypothesis. Postpartum-conceived litters may be female biased due to relative over-production of females at fertilization or shortly thereafter, and concurrent suckling stimulation of the mother may reduce this initial bias by selectively increasing female mortality during gestation. [Selective female mortality has been reported in mice (Krackow 1990); for a discussion of excess zygote production and hypotheses concerning the abandonment of excessive zygotes, see Kozlowski and Stearns 1989; Anderson 1990; Birney and Baird 1985]. This would result in a sex ratio closer to parity for postpartum-conceived litters that developed *in utero* concurrently with a large suckling litter. Confirmation of this mechanism must await experimental analysis that can vary systematically estrous condition and the number of pups suckling concurrently.

Nearly all studies of sex ratio in rodents have concentrated on litters conceived in cycling estrus. The results of the present study, however, suggest that more emphasis should be placed on litters conceived in postpartum estrus. Such increased emphasis is warranted given, for example, that approximately 50% of rat litters in the wild (Davis and Hall 1951) and 82% of litters born to monogamous pairs of house mice (*Mus musculus*; Bruce and East 1956) are conceived in postpartum estrus.

Finally, the changes in sex ratio that we have observed are interesting because they were achieved without the imposition of a stress procedure and without reductions in litter size. In contrast, stress-induced changes in sex ratio typically cause substantial reductions in litter size (e.g., Lane and Hyde 1973; Huck et al. 1988; Pratt et al. 1988; although this is not always the case; see Huck et al. 1986). Moreover, the variance in the sex ratio was less than expected from the binomial distribution. This reduced variance suggests that female rats have access to a mechanism for altering the sex ratio that they consistently called into play under adverse environmental or social conditions. This mechanism biases the sex ratio at birth without reducing litter size.

Such biasing of the sex ratio at birth could arise through several different selection mechanisms, affecting either the reproductive costs to the mother or the fitness of her offspring. For example: (1) Although a mother

that breeds asynchronously is at high risk of losing her offspring to inter-litter competition, this loss is not certain (Mennella et al. 1990). She may therefore conceive a litter, but minimize the cost of its potential loss by biasing the sex ratio towards daughters, the sex in rats that is "cheaper" to carry to term and raise to weaning (Hedricks and McClintock 1990; King 1915; Mennella et al. 1990; Moore and Morelli 1979). (2) Asynchronous breeding also signals that a mother will not have the opportunity to nurse communally and thereby share the high cost of nursing a postpartum litter (Mennella et al. 1990). Without this added support, she may reduce the cost of nursing by biasing her litter towards females, again because females individually cost less to raise. (3) Asynchronous breeding could also signal variables critical to the breeding success of her offspring, such as the sex ratio of mating adults or resource availability. If so, biasing the sex ratio of her offspring in response to this signal could increase her reproductive success, measured in number of grandoffspring. Each of these potential mechanisms must now be evaluated empirically in order to determine whether the sex-ratio biasing mechanism reported here confers an adaptive advantage or whether it is a by-product of another reproductive mechanism.

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References

- Altmann J, Hausfater G, Altmann S (1988) Determinants of reproductive success in savannah baboons (*Papio cynocephalus*). In: Clutton-Brock TH (ed) Reproductive success. University of Chicago Press, Chicago, pp 403-418
- Anderson DJ (1990) On the evolution of human brood size. *Evolution* 44:438-440
- Bertram BCR (1975) Social factors influencing reproduction in wild lions. *J Zool London* 177:463-482
- Birney EC, Baird DD (1985) Why do some mammals polyovulate to produce a litter of two? *Am Nat* 126:136-140
- Bruce HM, East J (1956) Number and viability of young from pregnancies concurrent with lactation in the mouse. *J Endocrinol* 14:19-27
- Calhoun JB (1962) The ecology and sociology of the Norway rat. (USPHS Publication No. 1008). Washington, D.C., US Government Printing Office
- Clark AB (1978) Sex ratio and local resource competition in a prosimian primate. *Science* 201:163-165
- Clutton-Brock TH, Albon SD, Guinness FE (1984) Maternal dominance, breeding success, and birth sex ratios in red deer. *Nature* 308:358-360
- Davis DE, Hall O (1951) The seasonal reproductive condition of female (Brown) rats in Baltimore, MD. *Physiol Zool* 24:9-20
- Enzmann EV, Saphir NR, Pincus G (1932) Delayed pregnancy in mice. *Anat Rec* 54:325-338
- Gilbert AN, Pelchat RJ, Adler NT (1980) Postpartum copulatory and maternal behaviour in Norway rats under seminatural conditions. *Anim Behav* 28:989-995
- Gudermuth DF, McClintock MK, Moltz H (1984) Suppression of postpartum fertility in pairs of female rats sharing the same nesting environment. *Physiol Behav* 33:257-260

- Hedricks C, McClintock MK (1985) The timing of mating by postpartum estrous rats. *Z Tierpsychol* 67:1–16
- Hedricks C, McClintock MK (1990) Timing of insemination is correlated with the secondary sex ratio of Norway rats. *Physiol Behav* 48:625–632
- Hrdy S (1987) Sex-biased parental investment among primates and other mammals: A critical evaluation of the Trivers-Willard Hypothesis. In: Gelles RJ, Lancaster JB (eds) *Child abuse and neglect: Biosocial dimensions*. Aldine DeGruyter, New York, pp 97–148
- Huck UW, Labov JB, Lisk RD (1986) Food restricting young hamsters (*Mesocricetus auratus*) affects sex ratio and growth of subsequent offspring. *Biol Reprod* 35:592–598
- Huck UW, Pratt NC, Labov JB, Lisk RD (1988) Effects of age and parity on litter size and offspring sex ratio in golden hamsters (*Mesocricetus auratus*). *J Reprod Fertil* 83:209–214
- Huck UW, Seger J, Lisk RD (1990) Litter sex ratios in the golden hamster vary with time of mating and litter size, and are not binomially distributed. *Behav Ecol Sociobiol* 26:99–109
- James WH (1979) The distributions of the combinations of the sexes in mammalian litters. *Genet Res* 26:45–53
- King HD (1915) The growth and variability in the body weight of the albino rat. *Anat Rec* 9:751–776
- Kozłowski J, Stearns SC (1989) Hypotheses for the production of excess zygotes: Models of bet-hedging and selective abortion. *Evol* 43:1369–1377
- Krackow S (1990) Sex-specific embryonic mortality during concurrent pregnancy and lactation in house mice. *J Exp Zool* 256:106–112
- Krehbiel RH (1941) The effects of lactation on the implantation of ova of a concurrent pregnancy in the rat. *Anat Rec* 81:43–63
- Labov JB, Huck UW, Vaswani P, Lisk RD (1986) Sex ratio manipulation and decreased growth of male offspring of undernourished golden hamsters (*Mesocricetus auratus*). *Behav Ecol Sociobiol* 18:241–249
- Lane EA, Hyde TS (1973) Effect of maternal stress on fertility and sex ratio: a pilot study with rats. *J Abnorm Psychol* 82:78–80
- Mantalenakis SJ, Ketchel MM (1966) Frequency and extent of delayed implantation in lactating rats and mice. *J Reprod Fertil* 12:391–394
- McClintock MK (1981) Simplicity from complexity: A naturalistic approach to behavior and neuroendocrine function. In: Silverman I (ed) *New directions for methodology of social and behavioral science*, Vol 8. Jossey-Bass, San Francisco, pp 1–19
- McClintock MK (1983) Pheromonal regulation of the ovarian cycle: enhancement, suppression and synchrony. In: Vandenberg JG (ed) *Pheromones and reproduction in mammals*. Academic Press, New York, pp 113–149
- McClintock MK (1984) Group mating in the domestic rat as a context for sexual selection: consequences for the analysis of sexual behavior and neuroendocrine responses. *Adv Stud Behav* 14:1–50
- McClure PA (1981) Sex-biased litter reduction in food-restricted wood rats (*Neotoma floridana*). *Science* 211:1058–1060
- Meikle DB, Tilford BL, Vesey SH (1984) Dominance rank, secondary sex ratio, and reproduction of offspring in polygynous primates. *Am Nat* 124:173–188
- Mennella JA, Blumberg MS, McClintock MK, Moltz H (1990) Inter-litter competition and communal nursing among Norway rats: advantages of birth synchrony. *Behav Ecol Sociobiol* 27:183–190
- Moore CL, Morelli GA (1979) Mother rats interact differently with male and female offspring. *J Comp Physiol Psychol* 93:336–341
- Myers JH (1978) Sex ratio adjustment under food stress: maximization of quality or number of offspring? *Am Nat* 112:381–388
- Packer C, Pusey AE (1987) Intrasexual cooperation and the sex ratio in African lions. *Am Nat* 130:636–642
- Pollard JH (1977) *A handbook of numerical and statistical techniques*. Cambridge University Press, Cambridge
- Pratt NC, Huck VW, Lisk RD (1988) Do pregnant hamsters react to stress by producing fewer males? *Anim Behav* 37:155–157
- Silk JB (1983) Local resource competition and facultative adjustment of sex ratios in relation to competitive abilities. *Am Nat* 121:56–64
- Smart JL, Dobbing J (1971) Vulnerability of developing brain. II. Effects of early nutritional deprivation on reflex ontogeny and development of behaviour in the rat. *Brain Res* 28:85–95
- Steiniger F (1950) Beitrag zur Soziologie und sonstigen Biologie der Wanderratte. *Z Tierpsychol* 7:357–379
- Telle HJ (1966) Beitrag zur Kenntnis der Verhaltensweise von Ratten, vergleichend dargestellt bei *Rattus norvegicus* und *Rattus rattus*. *Z Angew Zool* 53:129–196
- Trivers RL, Willard DE (1973) Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179:90–92
- Vorherr H, Vorherr VF (1984) Suckling-induced delay of implantation and increased fecundity in rats. *Gynecol Obstet Invest* 17:106–110
- Williams GC (1979) The question of adaptive sex ratio in outcrossed vertebrates. *Proc R Soc London Series B* 205:567–580