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Population Biology

Mother's prior intrauterine position affects the sex ratio of her offspring in house mice

(prenatal hormones/androgens/mammals)

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ABSTRACT **Sex ratio alterations related to environmental factors occur in several mammals, but no mechanism has been identified to explain the adjustment. Intrauterine position (IUP) may provide the context in which such alterations occur. Previous studies on house mice and gerbils reveal that the position of a fetus in the uterus in relation to the sex of its neighbors influences its later anatomy, physiology, and behavior. The anogenital distance (AGD) of females located between two males (2M) is longer than that of females not between two males (OM). We have found that the IUP, as determined by cesarean section and by an index of the AGD, correlates with the sex ratio of the litters produced by female mice. The sex ratio of the first litter born to 2M females was 58% males, for 1M females was 51% males and for OM females was 42% males. The effect on sex ratio continues into the second litter. The number of pups produced by mothers of different IUPs in her first two litters did not differ, suggesting that the sex ratio adjustment occurs prior to parturition. These results provide a basis for the natural variability observed in sex ratios of litter-bearing mammals and suggest that one or more intrauterine mechanisms may be responsible for environmentally related sex ratio alterations.**

Parental reproductive fitness would increase if the sex ratio of their offspring could be adjusted to relate to environmental conditions. Trivers and Willard (1) hypothesized that under favorable environmental conditions males would be favored and that under adverse conditions females would be favored in polygynous species because a few strong males could sire many grandchildren, whereas females have a more sure but limited reproductive potential.

A local resource competition model has been proposed (2, 3) to explain sex ratio alterations specifically for those species in which females show fidelity to their natal area and males leave the natal area to breed elsewhere. In this model, daughters of high-ranking mothers may achieve high reproductive success through inheriting the mother's high rank. However, since males breed in different groups, their reproductive success is presumably not influenced by the mother's rank. Thus, the local resource competition model predicts that, for certain species, high-ranking females would produce a female-biased sex ratio (3).

Among invertebrates, breeding adults can adjust the sex ratio of their offspring to maximize their fitness (4). Data from mammals have been more controversial. Clutton-Brock and Iason (5) review a number of studies conforming to a high standard of evidence and conclude that significant variation in sex ratios at birth in nonhuman mammals exists and that sex ratio varies with the costs or benefits of producing male or female offspring. Investigators have also shown that manipulating food can influence sex ratios at birth. Experimentally supplementing the food supply of wild opossums (*Didelphis marsupialis*) results in male-biased sex ratios (6), and restricting the food available to golden hamsters (*Mesocricetus auratus*) biases their sex ratio to females (7). The effect of food restriction on sex ratio is less clear in house mice. Females deprived of food on alternate days for 1 week produced female-biased litters, but similar deprivation for 2 weeks had no effect (8).

In addition to the physical factors, such as food supply, correlates of social rank may also influence the sex ratio of offspring. Recent work on domestic swine reveals that high-ranking females produce 59% males and low-ranking females produce 41% males (9). These data support the Trivers and Willard model for a species that, in the wild, is probably polygynous (10). On the other hand, Altmann (11) found that high-ranking female yellow baboons (*Papio cyanocephalus*) produced 34.5% male offspring compared with 68.2% for low-ranking females. These data support the local-resource model because in these nonhuman primates, females typically remain in the natal group and may inherit their mother's rank, whereas most males emigrate to other breeding groups. In rhesus monkeys, *Macaca mulatta*, females in lower ranking social groups and maternal geneology lines produce a lower proportion of sons than females in higher ranking groups (12, 13). When survivorship of groups are considered, high-ranking female rhesus monkeys had more surviving sons than did low-ranking females (14). Thus, considerable evidence exists that sex ratios in mammals can vary from the expected 50:50 ratio and that this alteration may relate to both physical and social factors in the environment affecting the parents.

A possible explanation for alterations in sex ratio over time is genetic selection. However, exhaustive attempts have failed to show that sex ratio can be altered by genetic selection. Breeders of domestic farm animals have been unable to vary sex ratio by selective breeding (4, 15) nor has genetic selection affected secondary sex ratios in house mice or the fruit fly, *Drosophila melanogaster* (16).

A recent finding by Clark *et al.* (17) suggests that another factor, prior intrauterine position (IUP), can influence the sex ratio in mammals that produce large litters. They report that female gerbils developing *in utero* between two males produce 57.1% males and that females developing between females produce 43.7% males. This finding suggests that factors present in the intrauterine environment can affect either the primary or secondary sex ratio. In rats and mice, IUP influences anogenital length (18, 19), central nervous system anatomical structures (20), reproductive physiology and behavior (21), and ecologically important variables such as home-range size in house mice (22). These effects are apparently due to the transfer of androgens from the male fetus to the female siblings. In this report we explore the

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Abbreviations: IUP, intrauterine position; AGD, anogenital distance; AGDI, AGD index.

possible effects of IUP on sex ratio in house mice (*Mus musculus domesticus*).

METHODS

Charles River CD-1 albino mice derived from our breeding colony were used. Animals were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication no. 86-23). They were maintained on a 14:10 light/dark cycle and provided with Prolab 3000 food and water *ad lib*. Females were mated when 60–90 days old with stud males, and the date of vaginal plug was noted. Half of the females were allowed to deliver vaginally, and the other half were delivered by cesarean section late on day 18 or early on day 19 of pregnancy. The normal gestation period for CD-1 mice in our laboratory is 19 days. Each pup was uniquely tattooed by subcutaneously injecting a droplet of India ink. The pups were gently cleaned, warmed, and given to lactating foster mothers. When 10 days old, each pup was toe-clipped based on the tattoo identification mark. This procedure was necessary to maintain identification of the known IUP females after the tattoo was covered by fur. Anogenital distance (AGD) was measured at birth for a portion of the females.

When weaned at the age of 21 days, the females from known IUPs were identified as 0M (not between two males), 1M (beside one male), and 2M (between two males) and were measured for body weight and AGD. AGD was measured with calipers from the base of the genital papilla to the proximal end of the anal opening. Care was taken to ensure that the skin of the anogenital region was not stretched or compressed. An AGD index (AGDI) was calculated as follows:

$$\text{AGDI} = \left(\frac{\text{AGD in mm at weaning}}{\text{weight in g at weaning}}\right) \times 100.$$

Use of the AGDI will be more fully described elsewhere (unpublished data).

Each animal was checked daily for vaginal opening, and when the vagina was open, a vaginal lavage was taken to detect cornification of the vaginal epithelium. Full cornification revealed the onset of first estrus (23, 24). The females were then isolated and bred with males from the 1M position when 3–4 months old. Body weight, sex, and AGD at birth were recorded for each individual in the litters produced by the known IUP females.

The females from the 0M and 2M positions were remated with a 1M male 15–30 days after weaning, and the resultant litters were analyzed as described above.

RESULTS

AGDI. A significant correlation was found between the AGD and the body weight of female mice at weaning (Fig. 1). Weanling females from the 0M and 1M IUPs showed an even distribution in AGD from 8 to 15 g (Fig. 1 *Top* and *Middle*) and a mean (±SEM) body weight of 12.24 ± 0.32 and 11.47 ± 0.25 g respectively, whereas those from the 2M IUP were clustered at low body weights (Fig. 1 *Bottom*). The mean body

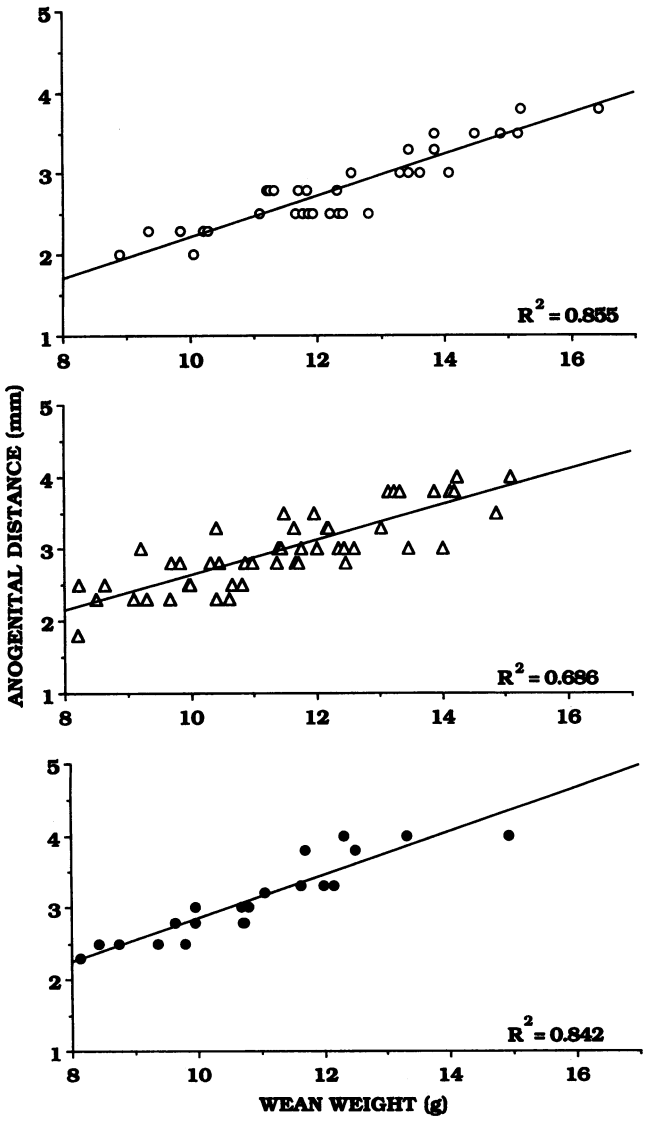

FIG. 1. Correlation between body weight and AGD at weaning of female mice derived from the 0M position (*Top*), the 1M position (*Middle*), and the 2M position (*Bottom*).

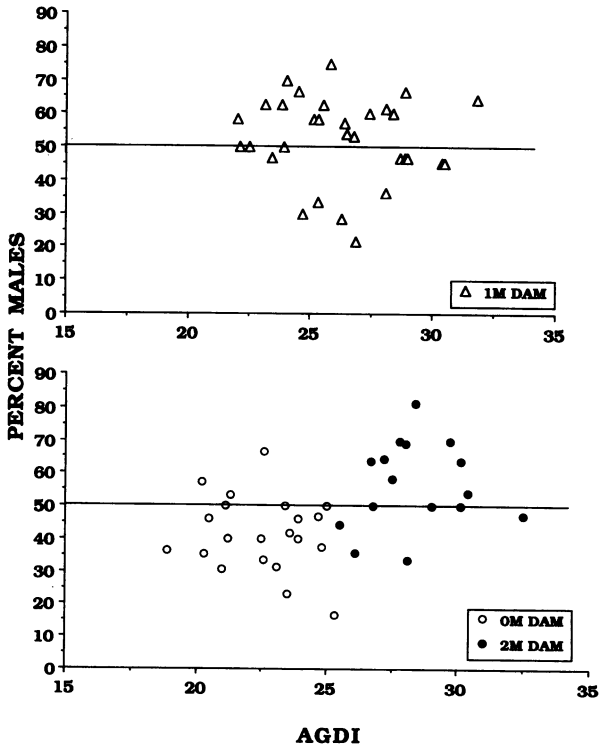

FIG. 2. Correlation between the AGDI measured at weaning of females from known IUPs as indicated and the percentage of males in their first litter.

Table 1. Percentage of males (mean ± SEM) in the first litters born to females from different IUPs when a single dam was selected from a central location in a uterine horn

IUP	n	% males*
0M	10	42.4 ± 2.61
1M	13	52.9 ± 2.14
2M	15	61.2 ± 3.24

*$P < 0.0002$ based on ANOVA. Mean values for 0M IUP compared with both 1M and 2M IUPs are significantly different at $P < 0.05$.

weight of the weanlings from 2M females (10.74 ± 0.37 g) was significantly lower ($P < 0.01$) than that of the weanlings derived from the other two IUPs.

Since a relationship exists between body weight and AGD, the AGD was adjusted for body weight to yield an AGDI for females from each known IUP. The AGDIs for 0M, 1M, and 2M females were 22.48 ± 0.31, 25.81 ± 0.36, and 28.38 ± 0.40, respectively. An overall ANOVA of the AGDI values across the three IUPs was statistically significant ($P < 0.0001$). Based upon the Tukey–Kramer test, the mean AGDI for females from each of the three IUPs differed significantly from each other ($P < 0.05$).

Sex Ratio. Examination of the sex ratios of the litters born to females from different IUPs revealed that 1M females produced a sex ratio of 51.4% males (Fig. 2 *Upper*), 0M females produced a sex ratio of 42.0%, and 2M females produced sex ratio of 58.5% (ANOVA, $P < 0.001$) (Fig. 2 *Lower*). A pairwise comparison shows that females in the 0M position delivered significantly fewer males than the females from the 2M position ($P < 0.008$); however, the sex ratio of litters born to 1M and 2M females did not differ ($P < 0.12$). Plotting the sex ratios against the AGDI of the females revealed that the 0M and 2M females cluster at the low and high ends of the AGDI, respectively, whereas the 1M females cluster around an intermediate position.

Since 2M females are never located at the ends of a uterine horn and no information is available on the consequences of such a position, we selected females from the center of the uterus and recalculated the sex ratios of the litters of females from each IUP. Thus, each 0M female was between two females and not at either end of the row in the uterus. Although the sample size was reduced, a highly significant sex ratio difference among females from different IUPs remained (Table 1). Using this analysis procedure also revealed that the sex ratio effect remained when a more stringent definition of sample independence was used.

The percentage of males in the litters born to females of known IUP was positively related ($P < 0.01$) to the mean AGD of the newborn females in that litter (Fig. 3). Females with the smallest AGD were members of litters with about 20% males, and females with the longest AGD were members of litters with about 70% males.

Second Litter. Females from the 0M and 2M positions were bred a second time with 1M males to determine whether the sex ratio effect would continue to appear in the second litter. 2M females produced two more pups per litter than 0M females in their second litter (Table 2). Sex ratios within the litters again differed significantly ($P < 0.04$) between 0M and 2M females (Table 2).

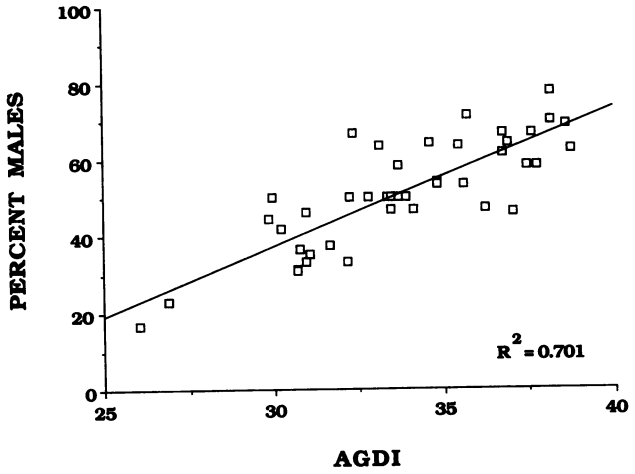

FIG. 3. Correlation between the percentage of males present in a litter and the average AGD of females in that litter. All litters were born to mothers of known IUP.

Combining the total number of pups for first and second litters reveals that the production of males in the first and second litters equaled 10.8 ± 0.53 for 0M females and 13.9 ± 0.91 for 2M females ($P < 0.0003$), a 22% increase in the number of males among 2M females. The total combined percentage of males for the two litters was 43.7% ± 1.80 for 0M females and 55.8% ± 2.44 for 2M females ($P < 0.02$).

DISCUSSION

The overall sex ratio of all of the litters examined in this study was 49.7%, close to the expected 50:50 ratio, but individual litters varied from 16.7% to 81.3% males. This variability is correlated with the prior IUP of the dams. Female mice derived from the 2M IUP produce a high proportion of male pups and 0M females produce a high proportion of female pups in their first and second litters. Females from the 1M IUP produce a balanced sex ratio in their litters. These results are remarkably similar to the prior finding of a correlation between IUP and subsequent sex ratio produced by female gerbils (17). In addition, we have shown that the AGD measured at weaning, the AGDI, correlates significantly with the sex ratio of the female's litters. This suggests that it may not be necessary to derive test mice by cesarian section for studies of prenatal hormone effects (unpublished data).

The finding reported here and the work of Clark *et al.* (17) suggest that a factor or factors derived from nearby male siblings *in utero* affect the sex ratio of litters born to polytocus mammals such as the house mouse and gerbil. The mechanism involved remains unknown, but one possibility can be rejected. Selective cannibalism by 2M and 0M females based on the sex of the pups could yield an adjusted sex ratio when litters are checked hours after birth. This possibility is

Table 2. Mean litter size and percentage males (±SEM) of first two litters born to females from known IUPs

	First Litter		Second Litter		Combination	
IUP	Litter size	% males	Litter size	% males	Litter size	% males
0M	12.36 ± 0.32	41.9 ± 2.47	12.63 ± 0.64	45.4 ± 2.21	24.0 ± 1.29	43.7 ± 1.80
1M	11.53 ± 0.25	51.4 ± 2.60				
2M	12.39 ± 0.37	58.5 ± 2.94*	14.63 ± 0.76	53.1 ± 2.85**	26.5 ± 1.66	55.8 ± 2.44**

The values presented for the combination of first and second litters represent only animals that had two litters ($n = 14$ litters for both 0M and 2M).
*$P < 0.001$.
**$P < 0.04$.

unlikely because litter sizes in the present study were not lower in the 2M and 0M females than in the 1M females and, more compellingly, litters of gerbils delivered by cesarean section and cross-fostered to lactating females showed the same IUP effect on sex ratio as those delivered vaginally (25).

Behavioral differences between the 2M and 0M females could be another possible mechanism to explain this effect. The 2M females took 5.3 days and 0M females 3.6 days to conceive, suggesting that the timing of insemination with regard to ovulation may have differed between the females. A curvilinear relationship between sex ratio and timing of insemination before and after ovulation seems to exist in rats (26) and humans (27, 28). This relationship results in more males being produced when insemination occurs either before or after ovulation and more females produced when insemination occurs close to ovulation. We did not monitor the ovarian cycle and thus do not know the date of ovulation in our mice but, since 2M females took 1.3 days longer to get pregnant than 0M females, it is likely that more females were inseminated separated in time from ovulation. Further support that mating times may differ comes from the finding that ovarian cycle length in 2M females mice is 1 to 2 days longer than in 0M females (29).

Another set of mechanisms available to explain the alteration in secondary sex ratio involve hormonal and biochemical events. These include: (*i*) factors influencing sperm transport, (*ii*) events occurring at or near the time of conception, (*iii*) zygote transport, (*iv*) implantation, (*v*) fetal resorption, and (*vi*) genomic imprinting. No information is yet available on these possible mechanisms or others as yet undiscovered. Any one or some combination of these mechanisms may be available to rodents or other mammals to adjust sex ratio.

In addition to alterations in the secondary sex ratio, species exhibit facultative adjustment of their litter's sex ratio. For example, eastern wood rat (*Neotoma floridana*) mothers provide equal suckling opportunity to male and female pups. But when food is severely restricted, mothers actively reject suckling attempts by males, thereby altering the sex ratio at weaning in favor of females (30). Such postpartum alterations of sex ratio may not be an adaptive adjustment of the sex ratio because the total reproductive output of the mother is reduced (31).

A relationship between sex ratio and IUP demonstrated here and by Clark *et al.* (17) provides a means whereby the production of one sex over the other can be increased to take advantage of environmental conditions that may enhance the survivability and reproductive success of one sex of progeny over the other. First, the natural variability in the sex ratio of litters produced by individual females may now be ascribed to the mother's prior IUP, and, since IUP is a random event, there will be a random distribution of the sex ratio bias. Second, the bias can become nonrandom if other factors, such as environmental stress, become additive or supplant the IUP influence on sex ratio. Such factors may operate through the same mechanism involved in the IUP-driven sex-ratio alteration.

Stress, both physical and social, is known to influence IUP-related anatomical, physiological, and behavioral effects in mice and rats. Earlier work on the consequences of physical stress, in the form of bright light, heat, and restraint applied to pregnant rats, resulted in reduced fertility of female offspring and feminization of male offspring (32, 33). Social stress produced by pairing female golden hamsters during pregnancy resulted in a reduction in the litter size and the sex ratio among subordinate mothers (34). Dominant females did not differ from unpaired control females. The alteration of sex ratio due to stress failed to appear following administration of dexamethasone, a synthetic glucocorticoid that inhibits the stress-induced release of corticotropin from the anterior pituitary (35). Litters of Norway rats (*Rattus norvegicus*) born asynchronously show interlitter competition that results in the litters becoming female-biased. Since this alteration in sex ratio occurs without reduction of litter size, it suggests that alteration of the secondary sex ratio occurred as a result of the competition (36). Experimental social stress can also influence a prosimian primate. Lesser mouse lemur (*Microcebus murinus*) females grouped up to the time of mating produced 67% males vs. 40% males for females kept in isolation prior to mating (37).

By applying physical stress to pregnant CF-1 house mice, vom Saal *et al.* (38) found that 0M and 1M female pups were masculinized and that all had AGD similar to those of 2M mice. Further, female pups from all three IUPs displayed elevated testosterone levels after application of maternal stress. The effects of stress can also result from grouping pregnant wild-type female mice with strangers. Such social stress resulted in all females having AGDs like those of 2M females (39).

The results reported here on mice and those of Clark *et al.* (17) on gerbils suggest that events during prenatal development can provide an explanation for the natural variability in sex ratio observed in litters. The proximity of a male fetus may be the primary variable affecting androgen exposure of nearby fetal siblings, but other sources of androgens are also possible. The possibility that maternal stress or environmental endocrine disrupters can interact with the IUP or directly affect the fetus to alter the sex ratio of her offspring provides a channel for environmental stimuli impacting pregnant females to influence the sex ratio of their progeny. This alteration can thereby affect population dynamics and, since maternal stress may be involved, may extend the sex ratio effect to mammals with small litters. These possibilities remain to be explored. The results presented here provide the context—fetal hormonal exposure—to explore the mechanisms whereby mammalian sex ratios can be adjusted to meet environmental challenges.

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