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Effects of Social Stress and Intrauterine Position on Sexual Phenotype in Wild-Type House Mice (*Mus musculus*)

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ZIELINSKI, W. J., J. G. VANDENBERGH AND M. M. MONTANO. *Effects of social stress and intrauterine position on sexual phenotype in wild-type house mice (*Mus musculus*).* PHYSIOL BEHAV **49**(1) 117–123, 1991.—Wild-type house mice were used to test the effect of intrauterine position on anogenital distance (AGD) and to verify whether crowding stress would masculinize female pups, developing at all intrauterine positions, as has been demonstrated in CF-1 mice stressed by restraint, heat, and light. Stress of crowding was documented by comparing aggressive behavior, litter birth weights, and plasma corticosterone levels among females in different densities. AGDs were recorded from pups born to females housed from day 12 to 19 of gestation either individually with their mate (nonstressed) or in one of two group-housed densities. Female pups from nonstressed dams positioned between two males in utero (2M females) had longer AGDs than females positioned between two females (0M females). AGDs of males from nonstressed dams did not differ on the basis of intrauterine position. Group-housed pregnant females in the higher of two densities had female pups with longer AGDs than female pups of other dams. However, variance in female pup AGD was no different among dams in different densities. These results extend to the wild house mouse previous findings in albino mice that intrauterine position influences sexual phenotype. In addition, social stress can induce masculinization of female pups in wild mice as physical stress has been shown to do in albinos. This suggests that intrauterine position effects and their modification by crowding stress can potentially influence the dynamics of wild house mouse populations.

Social stress Intrauterine position Sexual phenotype Mice

THE position of a fetus, in relation to the sexes of its neighboring intrauterine littermates, can influence its exposure to gonadal hormones and, therefore, its anatomical and psychosexual development (9,33). An individual located between two males (a 2M fetus) receives the greatest androgen exposure while an individual between two females (0M) receives the least; 1M fetuses receive intermediate levels of androgens (33). Prenatal hormonal environments influence several postnatal characteristics. For example, relative to 0M females, 2M female CF-1 mice have greater anogenital distances at birth, higher serum testosterone levels, are less attractive to males, have longer estrous cycles, produce less potent puberty-inhibiting pheromone, are less active, weigh more, are more aggressive towards other females, are more protective of their young, and learn shock avoidance less quickly (13, 16, 29, 31–36).

Sex determination and implantation location are random events, so the probability of a fetus occupying a particular intrauterine position can be calculated. For a litter size of 6, the relative frequencies of 0M, 1M and 2M individuals (including "terminal" pups at cervical and ovarian ends of uterine horns) are about 34, 50, and 16 percent, respectively (29). However, an important change in the characteristics of pups can be induced by stressing the dam (30,38). When restrained, pregnant CF-1 house mice are stressed by three daily exposures to high intensity light and heat, their 0M and 1M female pups are masculinized and have anogenital distances indistinguishable from 2M animals; they become "2M-like." Circulating testosterone in female pups from all three intrauterine positions increases in response to prenatal stress (38).

vom Saal (29) has suggested that the intrauterine position effect, and changes resulting from prenatal stress, may play a role in wild rodent population dynamics. He proposed that the variable intrauterine environment produced by differential proximity to males insures that female littermates will be sufficiently heterogenous in their physiology and behaviors so that some will be optimally adapted to any prevailing social environment. For example, 0M females have the advantage of being more attractive to males but the disadvantage of being less aggressive in encounters with other females than 2M females. Given the wide fluctuations that characterize house mouse populations (3,22), 0M females are predicted to have the greatest reproductive success in low density populations and 2M females will be more successful in high density populations.

The greatest affect on populations would be realized if *all females*, regardless of intrauterine position, behaved like 2M females when population density is high. If pregnant wild mice respond to social stress as CF-1 albino mice have responded to the stressors of light, heat and restraint, young females born into dense populations should exhibit the "2M-like" characteristics thought to be adaptive in crowded conditions. This idea can be considered an extension of the comprehensive work conducted by

Christian, Davis, and co-workers (6,19). The masculinization of female pups born to stressed dams is likely mediated by ACTH and glucocorticoid secretions (30) that are also implicated in the reproductive deficits and delayed development reported by Christian. Whereas Christian emphasized only the deleterious effects of stress on pups born in these conditions, vom Saal acknowledges possible negative effects (e.g., decreased immune response), but considers the 2M traits as adaptive in high populations.

To test the adaptive role of stress-induced changes in the intrauterine position phenomenon, we determined whether the "natural stress" of crowding would affect wild mice in the same way that light and restraint affect albino mice. During embryonic development perineal tissue responds to endogenous androgens such that short anogenital distance (AGD) identifies a pup as female (25). However, exogenous androgens, either from adjacent males or from the maternal circulation, can lengthen female AGD. Thus, AGD at birth is a useful assay of intrauterine androgen exposure by integrating endogenous and exogenous contributions, and is a good predictor of many adult behaviors influenced by prenatal androgen exposure (32,33).

In our study AGDs were measured in pups delivered from dams housed at different densities to determine whether 1) the basic intrauterine position phenomenon can be replicated in lab-reared wild mice under unstressed conditons, 2) AGDs are greater in female pups of dams that experience crowding stress (controlling intrauterine position) than in pups of unstressed dams, and 3) variation in the female AGD within a litter decreases with increasing crowding stress.

METHOD

Subjects, Housing and Mating

The subjects used were lab-reared descendants of wild mice caught near Alberta, Canada. All animals were kept on 14:10 light cycles in rooms where temperature was at approximately 23°C. Virgin females were time-mated with proven breeder males, had their pups removed immediately after parturition, and were bred during their first postpartum estrus by the same male. Because copulation verification (e.g., copulatory plug check, vaginal lavage for sperm) frequently causes embryo resorption in wild house mice (vom Saal, personal communication), the beginning date for the first pregnancy is only accurate to within the length of a female's estrous cycle. However, since females are usually impregnated within 18 hours of parturition, removing pups at birth assures that the next litter will be born 20 days later.

Experimental Design

On Day 12 of gestation, 58 pregnant females were marked with individually distinctive haircuts and assigned to one of three density treatments in $10 \times 13 \times 23$-cm cages. In the Low Density (Control) the female and her mate were merely transferred to a new cage. In the Moderate Density treatment the pregnant female, one unfamiliar male, and two unfamiliar females were grouped on Day 12 and a new unfamiliar female and male added to the cage on Day 15. Unfamiliar animals had never cohabitated with nor were closely related to the pregnant female or the other cage members and were added to assure that levels of strife remained high throughout the experiment. In the High Density treatment the pregnant female was grouped with two unfamiliar males and three unfamiliar females and an additional unfamiliar male and female were added on Day 15 and on Day 17. These procedures resulted in ultimate densities of 2, 6 and 10 animals per cage in the three treatments, respectively. All cages had ad lib food and water.

Between 1000 and 1200 h on Day 19 of gestation (conception equals Day 1) each female was removed from her cage and killed within 20 s by decapitation. Trunk blood was collected for corticosterone assay. Pups were delivered via caesarean section and their intrauterine positions were recorded. Each pup was resuscitated using vacuum suction and allowed to recover under a heat lamp for about 15 min. Pups were weighed, sexed and their AGDs were measured to the nearest 0.03 mm using an ocular micrometer. One observer, unaware of the intrauterine position, recorded all the AGDs. The AGDs of 22 of the pups (8 males and 14 females from 12 dams) were randomized and recorded a second time to produce a mean observer error of 0.044 mm (± 0.04 SD).

The following variables thought to influence anogenital distance were also included in the analysis: whether the pup was in a terminal position (immediately adjacent to the cervix or ovary), number of males in the specific uterine horn, proportion of pups in the horn that were male and, for 1M females, whether the adjacent male was on their cervical or ovarian side.

Indices of Social Strife

Behavioral. Aggressive and submissive behaviors were recorded by observing interactions in six of the High Density replicates and seven of the Moderate Density replicates (aggressive behavior was assumed to be negligible in the Low Density treatment). The number of Squeals, Submissive Postures, Chases (no bites delivered) and Fights (bites delivered) were recorded for fifteen minutes during two times of the day [0730–0830 (light) and 1730–1930 (dark)] on Days 13, 15 and 17 of gestation. Except for the pregnant female, the individuals involved in each interaction were not identified.

Pup birth weights. Stressing pregnant mice and rats with heat and light has been shown to decrease pup birth weight (14, 15, 40), though the influence of crowding stress on pup weight is not consistent (5,7). Given that mean pup weight may be a reliable index of stress during gestation we recorded mean litter weights from dams in each of the three densities. Litter size and sex ratio were also recorded since they have previously been shown to vary with maternal stress, in part due to sex-biased embryo reabsorption (11, 17, 21, 41).

Corticosterone concentrations. Corticosterone concentrations were measured from the plasma of pregnant females using radioimmunoassay (RIA). Corticosterone was extracted twice with 2 ml of an ethylacetate:chloroform (80:20) mixture, dried under nitrogen, and reconstituted in 140 μl buffer. It was necessary to further dilute maternal plasma (1:40) in buffer to obtain corticosterone concentrations that fell within the working range of the assay. Recovery was monitored by addition of 2800 dpm (5 pg) of [1,2,6,7-^{3}H]-corticosterone (85.8 Ci/mmol; New England Nuclear, Boston, MA) to quality control samples before extraction. A 20 μl aliquot for recovery and duplicate 50 μl aliquot for recovery for steroid radioimmunoassay were withdrawn after reconstitution. Recovery was routinely greater than 90%.

[1,2,6,7-^{3}H]-Corticosterone (85.8 Ci/mmol) was obtained from New England Nuclear, and unlabelled corticosterone was obtained from Steraloids, Inc. (Wilton, NH). Corticosterone antiserum (rabbit) was obtained from Dr. Gordon Niswender (Ft. Collins, CO). Cross-reactivity with progesterone was 0.39% and 0.44% with cortisol; cross-reaction with all other steroids was less than 0.1%. Duplicate aliquots of 50 μl of extracted steroid in buffer were incubated for 4 h at 24°C with 18,000 dpm (33 pg) of ^{3}H-corticosterone and antiserum to corticosterone (diluted 1:600). Free and bound steroids were separated by addition of second antibody (diluted 1:11; goat, anti-rabbit; Radioassay Systems Laboratories, Inc., Carson, CA). Tubes were incubated overnight at 4°C and then centrifuged at $1500 \times g$ for 1 h. The supernatant was

decanted, and the precipitate (representing the bound portion) was counted in a liquid scintillation counter (Beckman LS 5801, efficiency for tritium: 56%) after addition of 5 ml of Scintiverse II (Fisher).

The range of the standard curve for corticosterone was 60–2000 pg/tube. The blank of the assay was indistinguishable from baseline. The inter- and intraassay coefficients of variability were 11% and 2%, respectively. Charcoal-treated mouse serum was extracted and assayed as described above, and the corticosterone values were indistinguishable from the baseline of our assay. After addition of corticosterone (286 pg) to different volumes of charcoal-treated serum, we were able to measure 94% of the added steroid (means ± S.D. corticosterone concentrations, uncorrected for extraction losses, 277 ± 20, 258 ± 20, 267 ± 35 pg/tube, respectively), indicating that there were no effects of plasma volume on the concentration of corticosterone measured.

Statistical Analyses

All analyses of variance were conducted using the General Linear Model (GLM) procedure of the SAS statistical software program (SAS Institute, Cary, NC) and post hoc comparisons were made using the LSMeans procedure within GLM.

Intrauterine position (IUP) was considered a categorical variable since preliminary analyses showing no linear relationship between AGD and IUP precluded treating it as a covariate. Density and IUP were treated as main effects. IUP, Density, and IUP by Density interactions were assessed within litters, using Dam-within-Density error sum-of-squares. Examination of plots of residual vs. predicted values indicated that no transformations of the raw data were necessary.

The effect of IUP on AGD and weight was determined separately for the control group (Low Density) to compare our results with previous work with unstressed CF-1 mice. Because 2M pups were less common than 1M and 0M pups (10.9% of 412 pups were 2M), their inclusion in some analyses produced erroneous results due to nonorthogonality, so, for some Density analyses data from 2M pups were excluded.

Behavioral measures of aggression and submission were compared between Moderate and High Density treatments using analysis of variance. Density, Day (13, 15 or 17) and Time (a.m. or p.m.) were the main effects and the Density by Day and Density by Time interactions were of particular interest. Chi-square and F tests were used, respectively, to determine whether sex ratios differed from 50 percent and whether treatment affected sex ratio. Mean birth weights and log-transformed plasma corticosterone concentrations were subjected to analysis of variance and post hoc comparisons were made using the LSMeans procedure.

RESULTS

Social Strife Validation

Aggressive behavior. Aggressive behavior was greater in the High Density than the Moderate Density treatment (Table 1). Density had a significant effect on numbers of Squeals ($F = 8.53$, $p = 0.005$) and Attacks ($F = 12.30$, $p = 0.001$), and a nearly significant effect on number of Chases ($F = 3.55$, $p = 0.065$). Total Interactions (Squeals, Chases and Attacks combined) differed significantly between Moderate and High Density treatments ($F = 5.79$, $p = 0.020$). There was a significant effect of Time ($F = 5.89$, $p = 0.019$), a Time by Density interaction ($F = 7.05$, $p = 0.010$), and a Day by Density interaction ($F = 6.55$, $p = 0.003$, Table 2) on Total Interactions. These interactions indicate that, 1) mice were generally more aggressive during the dark than the light phase, 2) the difference in aggression between dark and light was more pronounced in High Density cages, and 3) mice in the two density conditions demonstrated different aggression levels on different days, with those in High and Moderate Densities most aggressive on Day 15 and Day 17, respectively.

TABLE 1

COMPARISONS OF MEAN (± 1 SE) SUBMISSIVE AND AGGRESSIVE BEHAVIORS PER 15-min SESSION BETWEEN ANIMALS IN HIGH AND MODERATE DENSITY TREATMENTS; ALL TIMES AND DAYS POOLED

	High	Moderate
Squeals	1.69 (0.48)	0.54 (0.25)†
Submissive postures	0.09 (0.06)	0.41 (0.21)
Chases	0.74 (0.25)	0.28 (0.14)
Attacks	4.91 (0.97)	1.49 (0.50)†
Total interactions*	4.60 (1.06)	2.18 (0.75)†
No. of interactions involving pregnant female	2.02 (0.66)	0.15 (0.07)†

*Squeals, submissive postures, chases and attacks combined.
†Significantly different ($p < 0.05$) than animals in the High Density Group.

The effect of crowding on the pregnant female stress response is best measured by noting the number of aggressive interactions in which the pregnant female was directly involved. Significantly more of the total number of aggressive interactions involved the pregnant female in the High than in the Moderate Density condition ($F = 9.09$, $p = 0.004$; Table 1).

Birth weight, litter size and sex ratio. The mean litter birth weights were significantly affected by Density ($F = 3.55$, $p = 0.035$; Fig. 1A). However, only the difference between Moderate and High Density treatments was significant ($p = 0.010$). When analyzed separately there was also a significant main effect of Density on female ($F = 3.77$, $p = 0.029$) and male ($F = 3.43$, $p = 0.040$) littermate body weights. Female pups from dams in High Densities were significantly lighter than those from Moderate ($p = 0.01$) and nearly less than pups from Low Densities ($p = 0.08$). Male littermates in High Densities weighed less than those in Moderate Densities ($p = 0.01$) but were no different from those in Low Densities ($p = 0.18$). Neither the Intrauterine position nor Intrauterine position by Density interaction were significant for female or male pup birth weights.

TABLE 2

EFFECT OF TIME [0730–0900 (LIGHT) OR 1730–1900 (DARK)] AND DAY OF GESTATION (13, 15 OR 17) ON MEAN (± 1 SE) TOTAL INTERACTIONS IN HIGH AND MODERATE DENSITY TREATMENTS

	High	Moderate
Time		
0730–0900	1.59 (0.64)	2.61 (1.19)
1730–1900	7.44 (1.74)	1.81 (0.98)
Day		
13	3.91 (1.33)	0.36 (0.20)
15	6.83 (2.66)	0.21 (0.15)
17	3.00 (0.92)	5.57 (1.80)

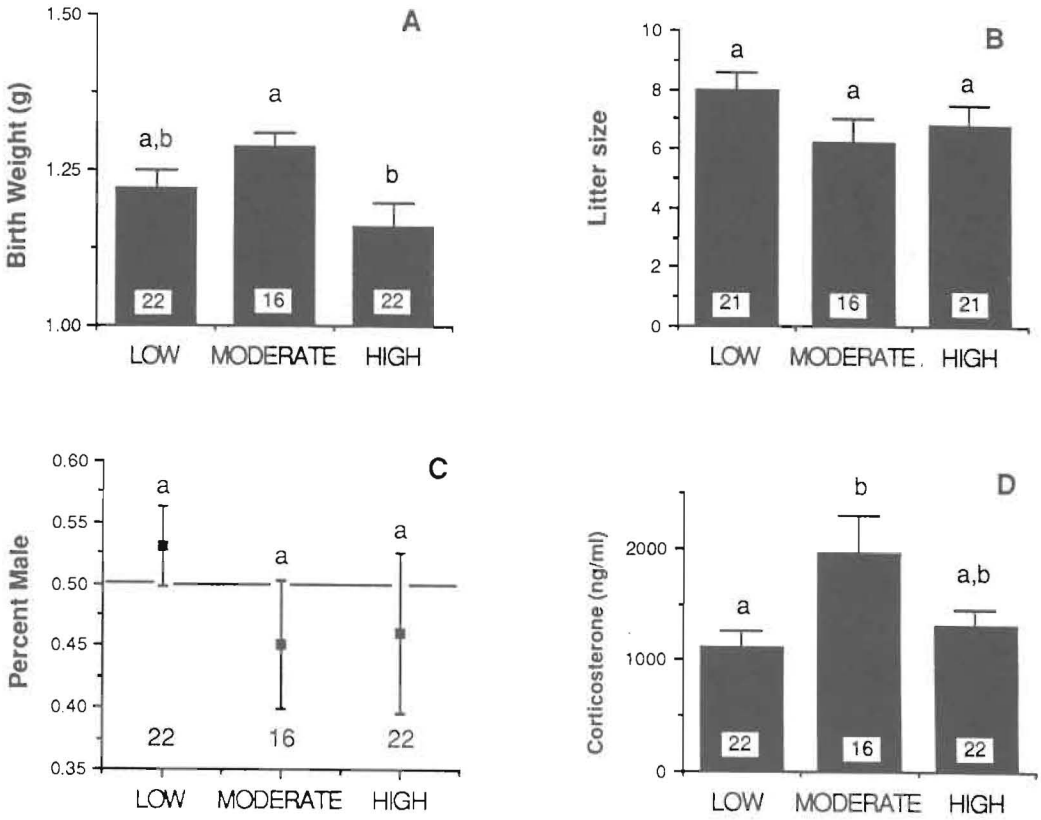

FIG. 1. Mean (±1 SE) (A) litter birth weights, (B) litter sizes, (C) sex ratios, and (D) corticosterone concentrations for dams in Low, Moderate, and High Densities. Number of litters are noted along the abscissa. Significantly different means ($p<0.05$) *do not* have the same letter above their standard error bars.

Density did not affect mean litter size ($F=1.77$, $p=0.18$; Fig. 1B). The percent (arcsine transformed) of the litter that was male did not differ among treatments ($F=0.30$, $p=0.742$) nor did chi-square tests detect a difference from 50 percent for any density ($p<0.05$; Fig. 1C).

Corticosterone concentrations. Mean log-transformed corticosterone values differed across treatments ($F=4.19$, $p=0.020$; Fig. 1D). Dams in Moderate Densities had significantly higher concentrations than those in Low ($p=0.043$), but High Density dams had levels no different from either Low ($p=0.17$) or Moderate Density dams ($p=0.43$).

Intrauterine Position and Density

Females. Before we compared the response of AGD to intrauterine position and density we simplified our analysis by determining that mean AGDs did not differ between terminal and nonterminal 0M or 1M female pups ($F=0.01$, $p=0.909$ and $F=0.13$, $p=0.725$, respectively) and that 1M pups with an ovarian-flanking male did not have longer AGDs than those with a cervical-flanking male ($F=0.06$, $p=0.802$). Therefore, subsequent analyses do not distinguish among different types of 0M or 1M females.

To test whether the basic intrauterine position phenomenon occurred in unstressed wild-type as well as laboratory mice (33) we analyzed data from females in the control (Low Density) condition separately. Intrauterine position had a marginal main effect on AGD ($F=2.60$, $p=0.083$) and consistent with previous findings in albinos the AGDs of 0M female pups were significantly shorter than those of 2M female pups ($p=0.027$; Fig. 2). The Dam effect accounted for the largest amount of variation in AGD ($F=5.62$, $p=0.0001$), indicating that individual differences in dams influenced the AGD of their pups more than the pup's intrauterine position.

When data from all pups born to dams in all three densities were included, AGD was not affected significantly by IUP ($F=0.38$, $p=0.684$). Dam again accounted for the greatest amount of variation in female AGD ($F=4.71$, $p=0.0001$). Although the Density by IUP interaction was significant ($F=3.57$, $p=0.014$), suggesting that the effect of IUP on AGD depends on the level of crowding, this result is probably an artifact of small 2M sample size, especially in the Moderate and High Density treatments (Fig. 2). Because all 2M pups from the Moderate and High Densities were from only 3 and 4 different dams respectively, we suspect that unbalanced sampling and a strong Dam effect rendered the IUP by Density interaction meaningless. Given this possibility, the analysis was run without 2M pups and resulted in neither a significant IUP nor IUP by Density interaction ($F=1.67$, $p=0.208$ and $F=1.88$, $p=0.171$, respectively). The 0M and 1M female pups did not have different AGDs nor did they respond differently to Density treatments and, therefore, the data from these pups could be pooled. The effect of Density on AGD was significant ($F=3.52$, $p=0.036$) and pups born to dams in High Densities had significantly longer AGDs than pups born to dams in Moderate Densities ($p=0.016$) and nearly significantly longer AGDs than those born to control dams ($p=0.086$).

Mean AGDs were also calculated for female pups in each uterine horn of each dam and related to the number and proportion of males in that uterine horn. Neither variable accounted for

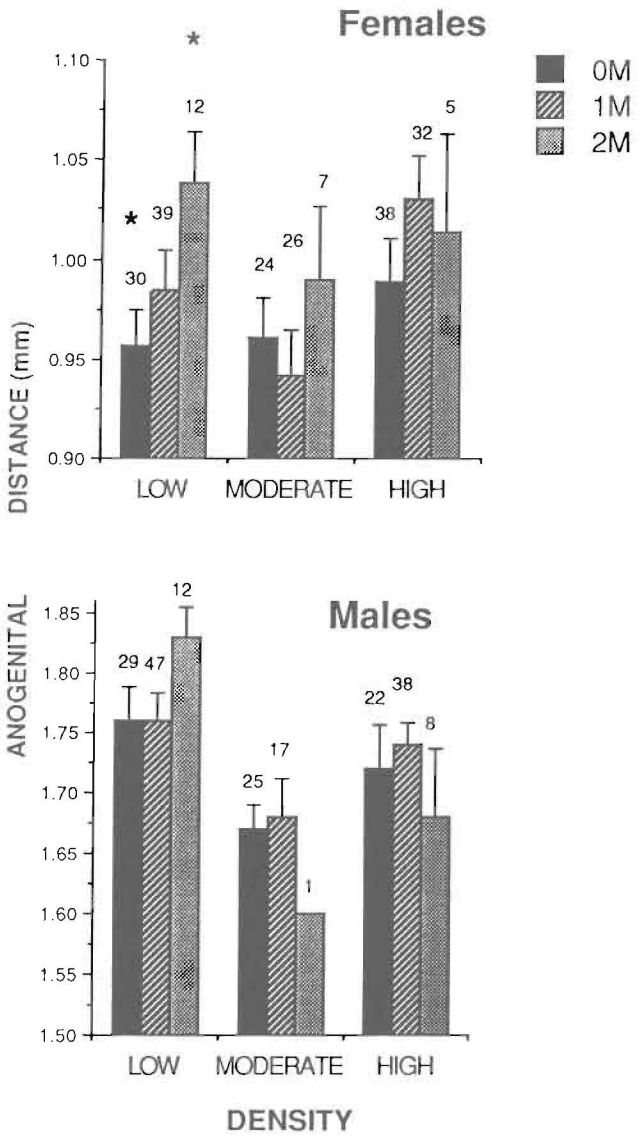

FIG. 2. Mean anogenital distances (±1 SE) for 0M, 1M and 2M female and male pups born to dams in different densities. The number of pups appear above bars. Asterisks denote IUPs, within densities, that are significantly different ($p<0.05$).

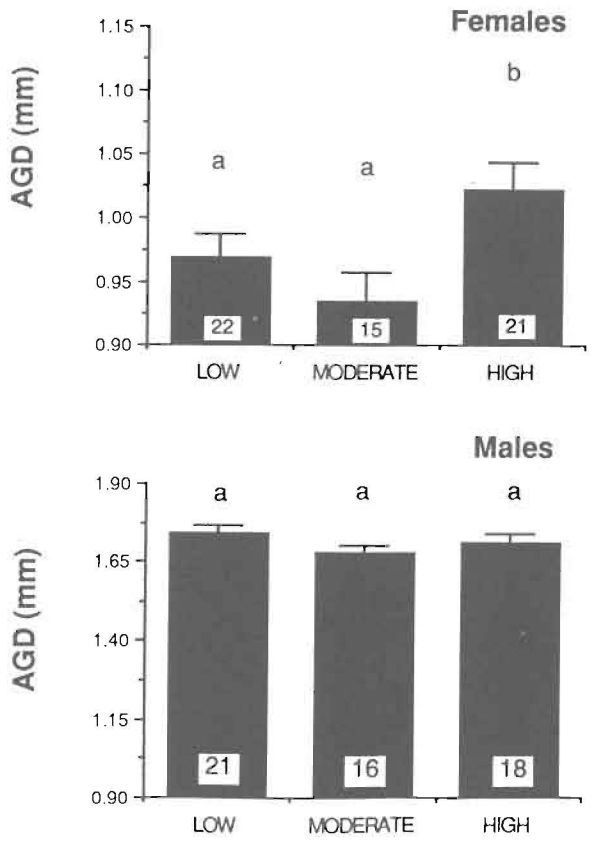

FIG. 3. Mean (±1 SE) of anogenital distances of female and male littermates born to dams in different densities; intrauterine positions pooled. Numbers imbedded in bars indicate the number of dams. Significantly different means ($p<0.05$) *do not* have the same letter above their standard error bars; Low vs. High difference for females is marginally significant ($p=0.06$).

a significant amount of variation in AGD ($F=0.62$, $p=0.434$ and $F=0.70$, $p=0.409$, respectively). However, consistent with the analysis by individual pups above, the Density effect was significant ($F=3.44$, $p=0.039$). Because the treatment, Density, was applied to the dam rather than to individual pups or uterine horns, the most appropriate test of the effect of Density on AGD is to compare means of female littermates across treatments. When this is calculated there is once again a significant Density effect ($F=4.14$, $p=0.021$). Female pups from High Density dams had significantly longer AGDs than those from Moderate Density dams ($p=0.007$) and nearly different AGDs from female pups from Low Density dams ($p=0.060$; Fig. 3).

Previous work with CF-1 mice showed that 0M and 1M pups from stressed dams had AGDs very similar to those of a control 2M female (30). If this were the case for our data, variance in AGD should be least for High Density pups and greatest for Low Density pups. Contrary to this prediction, Bartlett's test for homogenity of variances (27) indicated that there were no differences in the AGD variance among treatments ($\chi^2=0.21$, $p>0.05$).

Males. Considering only male pups born to dams in the Low Density (Control) treatment, there was no effect of IUP on AGD ($F=0.95$, $p=0.387$) nor were any of the post hoc comparisons among positions significant (Fig. 2).

Using individial pup AGDs as the dependent variable, analyses including all treatment densities indicated neither IUP ($F=0.61$, $p=0.547$) nor IUP by Density interactions ($F=0.16$, $p=0.959$) and no effect of Density on AGD ($F=1.16$, $p=0.321$). Additional analyses using mean uterine horn and mean litter AGDs showed that mean horn AGD bore no relationship to either the number ($F=1.52$, $p=0.225$) or the proportion of males ($F=0.87$, $p=0.358$) in the uterine horn. Further, neither mean horn AGD ($F=0.82$, $p=0.447$) nor mean litter AGD ($F=1.05$, $p=0.358$) were affected by Density. At neither the level of AGD of individuals, mean horn AGDs, nor mean litter AGDs did IUP or Density account for a significant amount of variance in AGD. Male pup AGDs, like females, were most influenced by their Dam ($F=4.31$, $p=0.0001$).

DISCUSSION

Our results suggest that the basic intrauterine position phenomenon, as demonstrated by genital morphology, occurs in fe-

male wild-type house mice. The 2M female pups born to unstressed dams had longer anogenital distances (AGDs) than did 0M female pups, a finding that generally agrees with work on CF-1 inbred mice (33). We found no differences between terminal and nonterminal pups, nor did we find evidence to support greater androgen exposure of 1M females with cervical-flanking rather than ovarian-flanking males (37). Only males immediately contiguous with a female influenced female AGD; neither the number nor the proportion of males in the uterine horn or litter affected AGD.

Particularly important to population ecology of mice are the effects that maternal stress may have on pup morphology, physiology and behavior. Female pups born to dams in the most crowded conditions had greater AGDs than those born to dams in the Low or Moderate Densities, however, the margin of significance suggests caution in the interpretation of the results. The mechanism for stress-induced masculinization remains unclear but the combination of an increase in maternally contributed testosterone, presumably from the adrenals and placenta (26), and the inhibition of fetal estrogen indirectly by maternal corticosterone has been suggested (30). However, corticosterone levels in female pups from stressed and unstressed dams do not differ (20). And, while elevated ACTH levels can increase testosterone directly in other species (10), recent results cast doubt on the direct role of estradiol or testosterone in stress-induced masculinization (38).

The work of vom Saal and Bronson is not completely supported by our data. They found that variance in AGD was reduced in pups born to stressed dams while we found no difference in AGD variance among treatments. Despite different means, the variances of pup AGD among High, Moderate, and Low Densities were no different. If stress lengthens AGD of all females to a distance equivalent to that in a 2M female, variance among pups within the High Density should have been less than that for pups in Low Density. Wild house mice may be more heterogenous in their response to stress than CF-1 mice or the level of crowding was not stressful enough to produce the maximum effect.

The finding that female pups born to moderately stressed dams had AGDs similar to pups born to control dams was surprising given that the pregnant females housed in Moderate Densities appeared to experience social stress above that in the Low Density treatment. The lack of effect on the AGD of the pups from Moderate Densities could be due to introductions of unfamiliar mice terminating on Day 15, before genital differentiation was complete (25). Thus, the higher social strife throughout the period of genital differentiation in High Density cages and the evidence of more direct involvement of the dam in conflicts in High Density cages may explain the lack of effect of Moderate crowding on female AGD. However, the high level of corticosterone concentrations in dams from Moderate Densities argue against this interpretation. Given the great variation within and among treatments, this difference may reflect the level of unquantified stress that females experienced in the day or so prior to plasma collection rather than integrating the stress response over the course of the seven day experiment. Plasma corticosterone levels are sensitive to acute stressors, habituate rapidly (23), and decrease to baseline usually within 24 h after acute stress in pregnant females (2,20). In the future, corticosterone should be measured throughout the duration of the experiment rather than only at the end.

The AGDs of male pups did not differ on the basis of intrauterine position or level of maternal stress. However, CF-1 0M male pups have higher estradiol-17β and lower testosterone plasma concentrations than 2M males, and a number of behavioral differences between adult 0M and 2M males have been reported (32). Males appear to be maximally masculinized since injecting a pregnant female with testosterone does not elongate the AGDs of her male pups (28). Thus, male perineal tissue is an exception to the generalization that "the organization of tissues that have androgen receptors, but not estrogen receptors, is enhanced in 2M male fetuses" (32). AGD appears to be a more useful assay of prenatal androgen exposure in female than male pups. It was also surprising to find that maternal stress did not affect male AGD, given that the testes of prenatally stressed rat pups produce less testosterone (39) and that previous reports indicate both shorter (1,40) and longer AGDs (18) in prenatally stressed male mice and rats. However, prenatally stressed males don't necessarily have altered sexual behavior (24). Either our crowding conditions were not sufficiently stressful to affect male AGD or it is unaffected by maternal stress.

The dam profoundly influenced the AGDs of females and males, indicating that pup AGD was influenced more by some unspecified characteristic of their mother than by intrauterine position or density. While evidence that maternal IUP can influence pup AGD is lacking, second generation effects of intrauterine stress on reproductive capacity have been demonstrated in house mice (7) and in gerbils (8). The importance of the dam effect is in agreement with the observation in voles that behavioral and physiological traits can be influenced more by maternal effects than by other factors (4,12).

Stress from crowding can affect demography in many ways. We have focused on whether stress can masculinize female pups of crowded dams and change the frequencies of different sexual phenotypes in a population. The intensity of social strife in the High Density condition may have been greater than that encountered in the field since defeated animals had no refuges and could not be driven out of the vicinity of the pregnant females. However, the rate of introduction of new individuals was probably similar to high density populations where social structure has eroded and transients are common (3). Provided our densities can be extrapolated to the field, females living in peak population densities can produce female pups with masculinized genital morphology that, presumably, will exhibit the adult behavioral and reproductive characteristics of 2M females. Whether this combination of attributes is adaptive is unknown; it may be a highly evolved system selected to fine-tune offspring to prevailing environments or it may simply be an artifact of maternal response to stress and occur without benefit, or may even be detrimental, to the offspring.

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