Urine Marking and Maternal Aggression of Wild Female Mice in Relation to Anogenital Distance at Birth

P. PALANZA,**1 S. PARMIGIANI AND F. S. VON SAAL

*Dipartimento di Biologia e Fisiologia Generale, Università di Parma, 43100 Parma, Italy. **Dipartimento di Scienze Ambientali, Università Ca' Foscari, 30187 Venezia, Italy and 1Division of Biological Sciences, University of Missouri-Columbia, Columbia, MO 65211 USA

PALANZA, P., S. PARMIGIANI AND F. S. VON SAAL. Urine marking and maternal aggression of wild female mice in relation to anogenital distance at birth. PHYSIOG. BEHAV. 58(4):200-200, 1995.—A series of experiments were conducted with wild house mice to verify the effect of intraspecific position on females' anogenital distance at birth (AGD) and to examine the relationships between a female's AGD used as a measure of estrogen exposure during fetal life, and her social behavior and reproductive success in adulthood. Experience I showed that cesarean-delivered females that developed in utero between two males (2 M females) have significantly longer AGD's than females positioned between two females (0 M females). We then categorized naturally delivered females shortly after birth as having a long, medium or short AGD. In adulthood, these females were tested for their behavior towards unfamiliar pups, their rate of urine-marking in response to a variety of social stimuli, parturition aggression and success in protecting their litters in response to male and female intruders. Adult females with different AGD's at birth did not differ either in their behavior toward pups or in their rate of urine marking. Conversely, males housed across a sire-sex partition from a long-AGD female purchased a higher number of urine marks than those exposed to a short-AGD female. When tested after delivering a litter, long-AGD females displayed more tail-rattling (a component of aggressive behavior) towards intruders of both sexes in comparison to short-AGD females. These results are consistent with the hypothesis that females with a long AGD are exposed to higher levels of gestational maternal estradiol than females with a short AGD. Although not related to AGD, other measures of maternal aggression were affected by parturition day, sex of intruders and a female's infantidal potential as virgin.

Urine marking  Wild female mice  Anogenital distance  Maternal aggression

IN THE house mouse, prenatal exposure to estradiol hormones is a potent source of variability for many aspects of a female's anatomy, physiology and behavior (30). The position of a fetus within the uterus, in relation to the sex of its adjacent littermates, influences its exposure to gonadal hormones and therefore its anatomical and psychosexual development, including aggressive behavior. A female house mouse located between two males (0 M) receives the greatest testosterone exposure, while an individual between two females (0 M) receives the least. 1 M females, between one male and one female, receive intermediate levels of testosterone and estradiol. Previous experiments on CF-1 mice have shown that, relative to 0 M females, 2 M females have greater anogenital distances at birth (AGD), are less attractive and preparing to mate, have longer estrus cycles, are less active, weigh more, and produce less potent puberty-inducing pheromones (reviewed in 29). 2 M female CP-1 mice are more aggressive toward other females than are 0 M females (30). However, in the study of aggression in mice, little attention has been paid to female agonistic behavior and its potential role in the regulation of social organization. Recent studies have shown that females compete among themselves for the opportunity to reproduce and can play an important role in determining social dynamics throughout aggression and infanticide (14,34).

Contrary to findings with domestic stocks of mice, most wild female mice exhibit infanticide as virgin. out this behavior is related to intrasexual competition for resources (9,12). Aggression by females when nursing young (i.e., maternal aggression) may influence the capacity for litters to be successfully defended against potentially infanticidal conspecics that intrude into the

---

1. References for reports should be addressed to Paris, Palanza. Dipartimento A Biologia e Fisiologia Generale, Università degli Studi, Viale delle Scienze, 43100 Parma, Italy. E-Mail: PALANZA@FPRPSO.UNIPRIET.
tory pit. Aggressive behavior by females put appears to be
related to spatial distribution and reproductive success (16,34). It
follows that factors affecting the development of a female’s
aggressive behavior toward conspecifics may be of importance in
determining her reproductive fitness as well as shaping social
structure.

The purpose of the first study was to determine (using a sock
of wild mice trapped in Canada) if intraspecific distance at birth
correlates with immature position, which correlates with prosta
tic exposure to testosterone (30,33). In the remainder of our
studies, the relationship between AGD at birth and a female’s
behavior in adulthood was studied; we categorized females as
having a long or short AGD at birth and then in adulthood
examined their behavior toward unfamiliar pups, their rate of
use, marking of a novel environment, or a variety of social
situations, postpartum aggression (after delivering a litter) toward
maternal and unfamiliar intruders, and success at priming their
young during lactation tests.

EXPERIMENTAL METHOD AND RESULTS

Animals

Rats were trapped near Cuyahoga, Akron, Ohio, and were main
maintained as an outbred colony at the University of Texas-Austin
and, subsequently, at the University of Missouri-Columbia. Ani
mal means were maintained at 25° C. in a 12:12 LD cycle, with
lights on at 0000 h. Water and food were available ad lib.

EXPERIMENT 1

RELATIONSHIP OF INTRASPECIFIC DISTANCE AT BIRTH TO
INTRAUTERINE POSITION

For production of asians from known intraspecific positions, 27
young-adult virgin females were paired with young-adult sexually
experienced males. On the day after a female delivered a litter,
the pups were removed and euthanized to facilitate labor-in
duced delivery and the postulation of pregnancy. In this study, if
parity occurs during the early portion of the light phase of the
dark-light cycle (prior to about L4000 h on the dark-light cycle),
pregnancy onset begins around the end of the light phase.
However, if parities occur later during the day, pregnancy
onset reliably occurs the following night. This finding, based on
preliminary studies, determined that if a female was
acquired to mate and thus determined when the subsequent litter
was determined on mating at pregnancy onset) was removed from
the study by cesarean delivery. We have found that females of this
date, as well as other (12), would stocks cannot be guaranteed for
the presence of a copulatory plug without inducing abortion (due to
stress). This technique thus allowed us to reliably determine
time from delivery to litter (on day 18 of pregnancy, with day
1 = mating; without handling the rat). Cataract delivery of pups
more than 12 h prior to normal parturition results in a high
rate of mortality.

Of the initial male and virgin female pairs, approximately two
thirds of the animals successfully produced a first litter and
then again, approximately two thirds of these animals produced a
second litter (n = 10 litters) which were removed during for
measurement of pups from different intraspecific positions.
Pups were delivered between 1000 h on Day 18 of pregnancy.
Pregnant females were killed by CO2 asphyxiation, and cervical
discharge, pups were rapidly removed. All of the intraspecific
position was recorded. Agemetric distance was mea
surement using an Olympus dissecting microscope with a
micrometer stage (accurate to 0.05 mm). Measurements were made
wet

Fig. 1. Mean (±SEM) intraspecific distance at birth of female pups from different intraspecific positions: 2 M vs. 1 M and 1 M vs. 0 M; p < 0.001 (M vs.
M, p = 0.05).

knowledge of intraspecific position by one person to whom pups
were passed. Body weight of each pup was also recorded. These
pups were placed with foster mothers but were not used for the
potential studies described here.

Intraspecific distance and body weights were measured for
different classes as occupying these different intraspecific positions: 0 M (between two female fetuses; n = 65); 1 M
(between two male fetuses; n = 50). With a litter size of 6-8, which is normal for this stock, on average, there should be one 0 M and one 2 M
around per litter, based on intraspecific position being a random event. This prediction is consistent with our results.
Intraspecific distance in males does not vary in relation to
intraspecific position (22) and is not reported here.

Data were analyzed using the Statistical Analysis System (SAS) multiple linear models. Data were analyzed by both ANOVA
and analysis of covariance (ANCOVA), with body weight used
at covariate, to determine whether portion of the variance in the
age differences in body weight. Planned comparisons were made using the Least Significance test.

The null hypothesis was rejected at the 0.05 confidence level.
Figure 1 shows the statistical (x 2) AGD in relation of each
mating position of females’ intraspecific position. There was a significant effect (p < 0.001)

of intraspecific position on AGD. M and 2 M females differed
significantly (p < 0.001), while 1 M females differed from 2 M females (p < 0.001) but not 0 M females (p = 0.08). There were
no significant differences between the groups in body weight. M
M = 1.18 ± 0.026; 1 M = 1.15 ± 0.015; 2 M = 1.10 ± 0.009.

ANOVA revealed that body weight accounted for a signifi
considerable portion of the variance in AGD (r = 0.01), but after being adjusted for body weight, mean AGD for 2 M, 1 M, and 0 M
females was visually identical to the mean presented above,
showing that differences in AGD occurred in a fashion of intraspecific position independent of a slight effect of body weight
on AGD.

We also examined two additional categories of females: those
occupying an intraspecific position at either end of an 8 mm (mean of 6.4 mm) or ovary of the Cr contemptuous group with an adjacent male fetus (1 M-end female) and an adjacent female fetus (0 M-end male).

PALAZZI, PAYNE, AND YOO ET. AL.
The mean ± SEM AGD for 1 M-en-d females (0.74± 0.015 cm; n = 26) was not significantly different from the mean AGD for 1 M females (0.75 ± 0.011 cm; n = 80). Similarly, the mean AGD for 0 M-en-d females (0.74 ± 0.014 cm; n = 35) was not significantly different from the mean for 0 M females (0.73 ± 0.012 cm; n = 60). However, body weights for both 0 M-en-d females (1.26 ± 0.03 g) and 1 M-en-d females (1.25 ± 0.03 g) were significantly (p < 0.03) greater than body weights for 0 M, 1 M or 2 M females, none of which differed at the end of a 20-week run.

The finding that females at the ends of the uterine horns are heavier than those in more central locations is consistent with prior findings that uterine horns receive the greater amount of blood flow (and thus nutrition). Blood enters the loop utricle feeding each uterine horn from each end of the loop (31). These findings show that proximity to uterine horns does not influence the AGD measure. Although comparisons of 2 M and 0 M males show that proximity to female uterine horns (and elevated estradiol) has effects on reproductive organs (29), also, the findings show that differences in AGD due to proximity to male uterine horns occur independent of effects on body weights of positions with the uterine horns relative to the utricle or cervix. It is thus inappropriate to present AGD data as a ratio of AGD/body weight (15).


d3.1

For gestational studies with female mice categorized on the day of natural birth as having a long, medium or short AGD, we used the female offspring of 1% pairs of wild mice bred at the University of Missouri. Within the first 12 h after natural delivery, pups were weighed, sex was determined, and the anogenital distance (AGD) of each female pup was measured in the nearest 0.01 mm using an Olympus dissecting microscope with a coupler micrometer. The distribution of the AGD measures for females used in this study is shown in Fig. 2. Female pups were marked by using a toe-clipping pattern to allow individual identification, and then they were returned to their mothers. Litters were culled to six pups by eliminating or adding male pups, but no litter had more than 4 female pups. After weaning, at 25 days of age, females were housed with same-sex littermates in polyethylene cages measuring 29 x 18 x 13 cm.

On the basis of findings from Experiment 1, and also by vent SacI & BclI (32) and vent SacI et al. (33), females with different AGD measures were separated into three experimental categories (Fig. 2). Short AGD (0.55-0.60 cm; n = 40). Medium AGD (0.60-0.80 cm; n = 129). Long AGD (0.85-1 cm; n = 50).

**EXPERIMENT 2**

**PRENATAL TEST FOR BEHAVIOR TOWARD MICE**

As 90-100-day-old females were housed individually, and 24 h later they were tested for their behavior toward a single 1-3 day pup. The pup was placed into a corner of with an adult's home cage with a minimum of disturbance. Females were scored as infants killed if they were unable to pick up the pup at which time the test was terminated; the pup was immediately removed and euthanized by CO2 asphyxiation. Females that did not attack the pups within 20 min were labeled as noninfanticidal. The uninjured pups were returned to their mothers. Throughout the study we took care to minimize the stress imposed on animals, both adults and infants. The number of pups used for infanticide tests was minimized by confronting adults with a single pup and removing the pups as soon as it was 

stacked (14).

Adult females with different AGD measures at birth did not differ in their behavior toward a newborn pup: approximately 5% of females exhibited infanticide, regardless of prenatal AGD.

**EXPERIMENT 3**

**ORNE MARKING BEHAVIOR**

Although females mark their environment a lower taxa than males, female uterine estrus may also play an important role in communication between female mice as well as in intersexual reproduction. It has recently been suggested that in natural populations of other small to medium-sized rodents, females mark in a more discrete, discrete territory during estrus. Moreover, since females from different intraspecific populations do differ in their attractiveness to and sexual arousal of males (24-30), the role of uterin marking by males when exposed to females with different AGD was also examined.

At 160 days of age females were individually housed for 24 h in polyethylene cages (29 x 18 x 13 cm) and then transferred to the test cages. Urine marking test were conducted for 1 h in cages (29 x 18 x 13 cm), divided into two equal chambers by a opaque, 0.5 cm wire-mesh barrier. The two sides of the chamber allowed visual, auditory, olfactory, and even tactile communication between animals in opposing chambers. The floor of the cage was covered by a large sheet of Whispaw No. 2 filter paper during the 1 h test.

The females were placed into the test cages and assigned to one of two experimental groups: 1) 12-long AGD females and 16 short AGD females were separated by the wire barrier; 2) a female of long AGD and a short AGD was separated by the wire barrier from a 90-day-old sexually naive male that had been individually housed for the previous 24 h. At the end
of the 1 hr urine-startling test, the filter paper was removed and 
describe urine marks (which fumed under uv light) deposited on 
rt by females or males were quantified (2). The mean number of urine marks deposited by females of 
short- and short-AGD was compared in the presence of a male of 
a female stimulus animal of the opposite AGD. Beating showed 
that female urine marking was not correlated with AGD whether 
females were housed across a wire partition (short short female 
or a male. However, both short- and long AGD females produced 
significantly more urine marks when in the presence of a male 
(about 50 marks/h) than when contacting a female stimulus (short 
marks/h: male vs. female stimulus for both long and short AGD 
females. P < 0.01).

Figure 3 shows that urine marking by males differed in the 
presence of long- vs. short-AGD females, with males exposed to 
short-AGD females depositing a higher number of urine marks 
than males exposed to short-AGD females (F test, F(2.8) = 2.8, 
P < 0.05).

EXPERIMENT 4
MATERIALS AND METHODS

During lactation female mice become intensely aggressive 
toward unfamiliar females that invade into the territory. This 
behavior, referred to as maternal aggression, may have 
evolved to protect the offspring from being killed by 
strangers. In fact, infanticide by both resident male and females 
has been well documented in house mice (e.g., 9,12,15). Material 
aggression appears to be a stereotyped phenomenon and can 
be categorized as either offensive or defensive, based on 
context and/or characteristics of opponent (16). However, infor 
mation concerning maternal aggression is based almost exclu 
sively on studies with domestic mice.

The primary study was to examine maternal aggression 
with mice in relation to AGD at birth. We also examined 
a number of other variables predicted to be correlated with 
maternal aggression, such as infant mortality tendency to be 
coming pregnant, length of time following delivery, and sex of 
the infant. Finally, we examined whether pregnancy causes, 
which occurs earlier during the first cycle, could affect 
behavior during delivery; would influence a female's behavior 
toward a male intruder, as well as the intruder's behavior towards the female 
and her pups.

Intruder females were a subset of those categorized as 
short-AGD at birth (Fig. 2) which had exhibited behavior 
during the preliminary test for her or his pups. The intruders were individually housed 3 days before being used 
in a test and were unfamiliar and generally uninterested to be 
interacting females (this was also true for intruder males).

Intruder males were sexually naive males of the same stock. After 
weaning the males had been housed in unique groups of 
4-6 individuals. At 90 days of age males were individually 
housed for 46 hr and tested for their behavior towards a newborn 
pup following the same procedure described for females. Only 
infrasexual males were used as intruders (13% of the tested 
males killed the pup). This test for infrasexual took place 3 days 
before the test during which the male was used as an intruder into 
the cage of a lactating female; all males remained individually 
handled until being used as intruders.

The experimental apparatus consisted of two perspex 
enclosures each (45 x 35 x 15 cm) connected by a 20-cm tunnel 
which could be closed by a removable partition. One of the 
tunnel was provided with a plexiglass box (10 x 7.5 x 7 cm) containing 
food material in which females could deliver their litters. The 
tunnel was covered by a removable transparent lid and fitted 
directly to the Aspin bedding. The intruder to the test box was 
provided via a 5-cm diameter metal which was 1 cm long. The 
tunnel was provided to simulate burrows or nests located in 
natural conditions and to provide the female a defensive nos 
trench. The second cage served to provide the intruder an area 
outside of the female's home cage in which to escape of attack 
by the female. The cage were covered by a stainless steel lid on 
which (used Pharaohs) and a square hole were placed.

Figure 1 shows that short- and long-AGD females 
pupared to attack adult males of 
the same strain background. A few days before delivery female mice 
were individually housed in the experimental apparatus. Per determining: 
both with male agp female intruders, a few minutes before 
introduction of an intruder, the lactating resident was 
confined in the cage containing the pup by inserting partitions or 
the female was moved to an adjacent cage. The intruder (on the back of a white mouse) 
was then placed into the cage not containing the pup. After 5 min with the pups were removed 
and the animals were allowed to interact.

Main Intruder Test

Twenty-two 1- and 9-AGD and 20 short-AGD lactating females 
were used for the experiment. One half of the long- and short- 
AGD females had shown infrasexual behavior in the present, while the 
remaining females had been reared invariability. Each female intruder 
went through 2 experimental tests on days 1, 2, and 4. 

This was done to examine the female's behavior towards 
male intruders during pregnancy and, subsequently, when 
maternal aggression is more intense (16). To maximize 
the likelihood of females being in parturition, intruder tests 
were conducted between 1700-2300 h on days 1 and 2 postpar 
. On day 4 postpartum test took place between 1900-1600 h. 

Details of observations and methods are described 
in detail, but the animals were continuously monitored to check for 
the occurrence of infanticide during the following 20 min. In
On day 4 postpartum between 1700 and 2300 h, a randomly selected subset of lactating females (19 short-AGD and 8 long-AGD) were tested for maternal aggression against a previously infanticide, medium-AGD female intruder. For each female, there was at least a 5-h period between the earlier test with the male intruder and the test with the female intruder.

Behavioral Analysis

The following behaviors of lactating females were recorded using an Esterline Angus multi-channel event recorder: (i) Proportions of initial attack; (ii) Latency to attack; (iii) Cumulated attacking time (i.e., the total duration of biting attack); (iv) Tail rattling (i.e., the total duration of tail lateral quivering or thrashing of the tail); (v) Fear or flight behavior (i.e., total duration of fear-related behaviors, such as contractile immobility, upright defensive posture, vocalizations and startle responses); (vi) Social investigation (i.e., total duration of sniffing and greeting the intruder); (vii) Mani- tance behavior (i.e., the total duration of self grooming, feeding and drinking); (viii) Nesting behavior (i.e., total duration on nests, crouching over pups, suckling nesting Cairn material); (ix) The total duration of non-social activities (i.e., exploration of the cage).

In order to assess whether females would differ in their success in protecting their litters as a function of their AGD at birth, the occurrence of, and latency to, infanticide by male intruders, as well as cooperation or competition by the male attacks by the lactating female resident, were recorded. Because a test session was immediately terminated if the intruder attacked a pup, the total duration of test sessions could vary. Thus, duration data for all behaviors were calculated as a ratio where the time (in s) spent in a given behavior was divided by the total duration of time of recording behaviors, which could be a maximum of 600 s. This ratio was then converted to percent (% x 100) so that the data for each behavior represented the percent of the test session engaged in the behavior. The total percent time engaged in the behaviors that were recorded was less than 100%, reflecting the fact that there were periods during which the test animal was immobile.

Chi-square analysis was conducted to compare the proportion of animals that exhibited a behavior. Latency measures were analyzed by nonparametric tests (i.e., Kruskal-Wallis and Mann-Whitney), since latency data were not normally distributed. Duration data for behaviors were analyzed by ANOVA using the two-factor, repeated measures analysis available on the Statistical Analysis System (SAS) general linear model.

Following the first test on day 1 postpartum, some females ceased to care for the pups, and in some cases the entire litter disappeared. The loss of litters occurred between the end of testing on one day and the time that animals were examined on the next day; in no case was this observed to occur by an experimenter. Maternal behavior of wild-type females is very sensitive to any disturbance, especially during the first 24 h following parturition (12). As a consequence, the number of experimental subjects on the different test days postpartum varied (i.e., short-AGD females): N = 22, 17, 16, long-AGD females: N = 20, 19, 17, on days 1, 2, 4, postpartum, respectively.

RESULTS

Only the data obtained on day 4, when both male and female intruders were tested, are presented in Table 1. With regard to differences based on postpartum day of testing (data not shown),

<p>| TABLE 1 | BEHAVIORS OF LONG- AND SHORT-AGD LACTATING FEMALES IN RESPONSE TO MALE OR FEMALE INTRUDERS |
|---------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Sex of intruder</th>
<th>Long AGD</th>
<th>Female</th>
<th>Male</th>
<th>Short AGD</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of attack</td>
<td>0.15(0.60)</td>
<td>0.15(0.60)</td>
<td>0.15(0.60)</td>
<td>0.15(0.60)</td>
<td>0.15(0.60)</td>
<td>0.15(0.60)</td>
</tr>
<tr>
<td>Latency to attack</td>
<td>2.5(1.07)</td>
<td>7.0(2.40)</td>
<td>12.0(4.00)</td>
<td>12.0(4.00)</td>
<td>7.0(2.40)</td>
<td>2.5(1.07)</td>
</tr>
<tr>
<td>Attack</td>
<td>4.8 ± 1.5</td>
<td>8.6 ± 1.6</td>
<td>4.8 ± 2.4</td>
<td>4.8 ± 2.4</td>
<td>8.6 ± 1.6</td>
<td>4.8 ± 2.4</td>
</tr>
<tr>
<td>Tail rattling</td>
<td>7 ± 5.1</td>
<td>4.8 ± 2.2</td>
<td>4.8 ± 2.2</td>
<td>4.8 ± 2.2</td>
<td>4.8 ± 2.2</td>
<td>4.8 ± 2.2</td>
</tr>
<tr>
<td>Fear or flight</td>
<td>1.6 ± 0.7</td>
<td>0.1 ± 0.07</td>
<td>3.7 ± 0.9</td>
<td>3.7 ± 0.9</td>
<td>0.1 ± 0.07</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td>Social investigation</td>
<td>0.1 ± 0.13</td>
<td>1.4 ± 0.5</td>
<td>0.3 ± 0.06</td>
<td>0.3 ± 0.06</td>
<td>1.4 ± 0.5</td>
<td>0.1 ± 0.13</td>
</tr>
<tr>
<td>Maintenance</td>
<td>3.7 ± 0.7</td>
<td>3.7 ± 0.7</td>
<td>3.7 ± 0.7</td>
<td>3.7 ± 0.7</td>
<td>3.7 ± 0.7</td>
<td>3.7 ± 0.7</td>
</tr>
<tr>
<td>Iron Social</td>
<td>10 ± 11.8</td>
<td>96 ± 11.8</td>
<td>96 ± 11.8</td>
<td>96 ± 11.8</td>
<td>96 ± 11.8</td>
<td>96 ± 11.8</td>
</tr>
<tr>
<td>Nest</td>
<td>2 ± 0.8</td>
<td>1.3 ± 0.8</td>
<td>7.4 ± 1.4</td>
<td>7.4 ± 1.4</td>
<td>1.3 ± 0.8</td>
<td>2 ± 0.8</td>
</tr>
</tbody>
</table>
the proportion of long- and short-AGD females attacking, and the time spent in different behaviors during the test with a male intruder, did not differ significantly as a function of day postpartum or for (i) tail-rattling; (ii) fear/defense; (iii) nest orientation; (iv) maintenance; and (v) non-social behaviors. In contrast, between days 1 and 2 vs. day 4 postpartum, the total duration of attack behavior significantly increased (by over 30%); P < 0.01). Fig. 4) while social investigation significantly decreased (by 85%; P < 0.01). Fig. 5) for both long- and short-AGD females. Latency to attack also decreased significantly for both long- and short-AGD females from day 1 and 2 to day 4 (by 90%; P < 0.05). Long-AGD females displayed significantly more tail-rattling toward male intruders than short-AGD females on postpartum days 2 and 4 (P < 0.03; see Fig. 6 for data on postpartum day 4).

Contrary to what is typically observed in domestic stocks of mice (such as the Rockland Swt 26), where very little aggression is observed until day 1 postpartum, wild-type females showed a relatively high rate of attack even during the first 48 h following delivery, when postpartum stress typically occurs. Attacks by females toward male intruders were commonly accompanied by vocalizations (typically associated with fear) and defensive postures. Bites directed toward the intruder's body were rarely vicious, since females often were observed initiating attacks but without actually biting the male. The resident females often remained at the entrance tunnel leading to the nest box, lunging towards the male, but typically tried to enter the nest box.

There was no relationship between a female's AGD and success at protecting her offspring from male infanticide. In spite of maternal attacks, the majority of males succeeded in entering the nest box and attacked a pup. If the test had not been immediately terminated at this time, prior studies using both Swiss (C57) and wild-type (SW) mice have shown that the entire litter would have been killed in every instance once an attack on one pup is initiated. Since the test was terminated at this time, there was no basis for assessing whether females with long- or short-AGD's might have differed in the likelihood of protecting and rearing at least some of their offspring once a pup was attacked.

**FIG. 4.** Accumulated attacking time (M ± SE) toward male intruders by lactating females tested on different postpartum days. ANOVA; AGD: ns; day: F = 5.4 p < 0.05; AGD×day: ns.

**FIG. 5.** Social investigation (M ± SE) of male intruders by lactating females of short or long AGD on different postpartum days. ANOVA; AGD: ns; day: F = 3.4 p < 0.05. AGD×day: ns.

**FIG. 6.** Accumulated attacking time (M ± SE) toward male intruders by lactating females with different potential for infanticide as virgins.

This is the legend of FIG. 7
For day 1 postpartum, about 25% of males attacked a pup within the first 10 min of observation, and about 75% of males attacked a pup within the 1 h test period; similar results were observed on test days 2 and 4. These data suggest that maternal attack was rarely successful in preventing male infanticide, although the complex housing condition employed (with two connected cages and the poisoned location of nest) could provide a relatively defended nest area and an environment into which the male could escape when attacked, in comparison to the usual laboratory test performed in one small cage.

The data in Table 1 show differences in the behavior of lactating long- and short-AGD females according to sex of intruder. Fem-male defense behavior was significantly influenced by the sex of intruder for both long- and short-AGD females, with both categories of lactating residents showing morerear and/or female behavior when confronted by male than female intruders (P < 0.001). A significant effect of the sex of intruder was found with regard to social investigation, with lactating females showing more social investigation toward female than male intruders (P < 0.001).

For both long- and short-AGD females, the latency to attack was shorter toward male than female intruders (P < 0.001). Females of long- and short-AGD did not differ in terms of the amount of time spent attacking the intruders, but with intruder females, there was a significantly greater total duration of attacks by both long- and short-AGD lactating females than was observed with intruder males (P < 0.05). Female intruders were often severely injured, whereas males rarely showed visible wounds.

Due to the very high rate of attack, toward the female intruders, we decided not to extend the observation beyond the initial 15-min period during which behaviors were recorded in order to determine whether infanticide would occur. However, given the intensity of attack toward female intruders, infanticide by the intruder females would have been unlikely, because the intruder probably would have been killed.

Figure 6 shows that there was significantly more tail rabling by long- than short-AGD females in response to both male and female intruders on Day 4 postpartum (P < 0.05). All females had been tested for infanticidal behavior as virgins, and as mentioned previously, this behavior did not relate to AGD. However, we explored whether there was a relationship between behavior toward a pup while a virgin and postpartum aggression toward male intruders. Lactating females that had been infanticidal as virgins showed a greater total duration of attack toward male intruders than noninfanticidal females (Fig. 7). No difference in attack toward female intruders on day 4 postpartum as a function of being infanticidal or noninfanticidal as virgin was found.

GENERAL DISCUSSION

The results of Experiment 1 showed that infanticidal position is correlated with anginal distance (AGD) at birth in wild female mice and that anginal distance at birth can thus be used as a bioassay for prenatal exposure to testosterone. Recently, Vuckiner and Huggett (personal communication) have extended this observation and found that anginal distance at weaning (corrected for body weight and referred to as the anginal distance index) can also be used as a predictor of fetal testosterone exposure; female mice categorized at pregnancy having a different anginal distance index differ in the age at first estrus and in the sex ratio of the first two litters produced.

The results of Experiment 3, in which females categorized at birth as having long- or short-AGD were compared in adulthood, showed that males housed across a wire mesh partition from a long-AGD female deposited a higher number of urine marks than those exposed to a short-AGD female. Conversely, female urine marking was not related to AGD whether females were exposed to a male or a female stimulus animal of the opposite AGD. These results are consistent with the hypothesis that females with a long AGD are more androgynized than females with a short AGD with regard to the release of phenomonal cues relative to short-AGD females (1). In this view, long-AGD females would simulate male courtship behavior-marks as a response to phenomonal cues produced following the exposure to a higher level of testosterone during fetal life and thus associated with male-type stimuli. Males tend to urine marks at higher rates when in the presence of a strange male as opposed to a strange female (1). Male urine marking may provide females with the opportun- ity to assess male social status, since females can discriminate male social status on the basis of urinary cues (8) and prefer to mate with dominant males (6,22).

Regardless of their AGD at birth, females produced far more urine marks when in the presence of a male than another female. This confirms previous results of Marunik et al. (11) with laboratory mice and recent data of Faurot et al. (18) on wild mice. Increased urine marking in response to male cues may function to advertise territory occupancy to other females, thus suggesting that male cues can stimulate competitive among females (10). While in CF-1 mice, 2 M females were found to urine mark at higher rates than 0 M females (30), in this experiment AGD at birth did not predict rates of urine marking. It is thus possible that in this wild stock of mice, prenatal exposure to androgens does not affect this behavior in females, even though it appears in

FIG. 7. Amount of tail rabling (M ± SEM) displayed by lactating females characterized by long or short AGD towards male or female intruders.
affect the prediction of pheromonal cues that stimulate utricling marking in males. The relative importance of intrasexual position phenomenon in infesting a variety of behavioral and physiologic elements may, in fact, vary in relation to the gene pool of a particular mouse population (25,35).

Experiment 4 showed that if delivering a litter, long-AGD females displayed more tail raking (a composite of mouse agonistic behavior) towards intruders of both sexes in comparison to short-AGD females. However, other measures of aggression by lactating female towards intruders of either sex were not related to AGD as both Aggressive females, as well as females, had been shown to be larger to expression of intrasexual behavior towards utricling during lactation in laboratory stocks of mice (27).

Specifically, previous studies have shown that 2.5 g female mice exhibited more intense aggressive behavior when nursing young than did 0.5 g females (10,30). Again, genetic difference can account for the discrepancy between previous findings on domestic mice and those on a wild stock with regard to the intensity of aggression.

Contrary to what is commonly observed in most laboratory stocks of mice in which little or no aggression is observed 2-3 days postpartum (20), in this experiment we observed that lactating females displayed relatively high rate of attack toward male intruders during the 24-48 h after delivery, which encompassed the period of post-partum estrus. However, the behavior of the lactating female toward male intruders did vary between days 1 and 2 and day 4 postpartum in that the intensity of attack increased while social investigation decreased.

Agonistic distance in birth did not release either to a females' infanticidal potential when virgin or to the intensity of aggression displayed by females when lactating. Nevertheless, a relation between these two behavior was found, at litters which were infanticidal as virgins were more aggressive toward male intruders than noninfanticidal females. Aggression toward adults and infanticidal behavior when confronted with a newborn pup can both be viewed as forms of intraspecific competition. Levels of different forms of aggression, such as intrasexual aggression, infanticide by males or females, and maternal aggression, covary within and between different lines and strains of mice, thus suggesting that these different forms of aggressive behavior can be a trait related to level of intraspecific competition within a population (20).

A major difference between the present study and experiments on laboratory stocks of mice is that both male and female intruders present a potential threat to the female's offspring. Aggression by lactating female mice toward other females, as well as males, can thus be a critical behavior to ensure survival of the litter. In accordance with previous studies on the laboratory Swiss line (21), wild lactating females respond differently to intruders of differing sex, displaying more social investigation toward female but more for related behaviors toward male intruders. Contrary to what is observed in laboratory stocks (13,21), attacks on females were more intense and relentless than the attacks on male intruders. None of the female intruders exhibited infanticide during maternal aggression tests, whereas most of male intruders attacked the lactating females and the pups. Maternal aggression thus appears to be successful in defeating, and clinging infanticide by other females but log by males. This finding, which confirms previous studies (e.g., 3,16,23), questions whether maternal attack on male intruders serves only as a counterstrategy to infanticide. It has been proposed that maternal aggression towards males could also serve to assess the quality, in terms of fighting ability, of males that will become the female's future mates (16,17,23).

ACKNOWLEDGMENTS

This research was supported by Italian CNR and MURST. NATO GRANT CRG 921382 to F.S. and S.P. and NIH grant AG-11314 to F.S.

REFERENCES

14. Palanza, P., Brown, P. F., Pandughi, S. I. Allostimpression in male and female intruders in a cartoon of infanticide. It has been proposed that maternal aggression towards males could also serve to assess the quality, in terms of fighting ability, of males that will become the female's future mates (16,17,23).

This research was supported by Italian CNR and MURST. NATO GRANT CRG 921382 to F.S. and S.P. and NIH grant AG-11314 to F.S.


