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Proximate and ultimate causes of infanticide and parental behavior in male house mice

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INTRODUCTION

The hypothesis that infanticide (i.e., the killing of conspecific young) is adaptive in some situations remains controversial. More generally accepted is the proposition that the killing of a member of one's own species is maladaptive. Infanticide has therefore been regarded as a pathological behavior that only occurs in response to a breakdown in social structure (Calhoun, 1962; Curtin and Dolhinow, 1978). Although a breakdown in social behaviors and an increase in both infanticide and aggression between adults certainly occurs during times of social stress (Lloyd and Christian, 1969; Christian, 1971), this does not rule out the possibility that in other circumstances, infanticide may be adaptive. Hrdy (1979) has proposed four sets of circumstances under which infanticide would be adaptive. Her classification is based upon the nature of the benefit (mates, food resources, etc.) to the infanticidal animal (see Introduction, this volume). In this chapter, only one category of infanticide is discussed: that relating to sexual competition between males, which is referred to as "sexually selected" infanticide. Traits that enhance the ability of individuals to compete with members of the same sex for the chance to reproduce are thought to be subjected to sexual selection, which Darwin distinguished from natural selection (cf. Trivers, 1972).

Three predictions generated from the hypothesis that infanticide

evolved through sexual selection (Hrdy, 1979; vom Saal and Howard, 1982) are that: (1) There must exist mechanisms to inhibit males from killing their own offspring; (2) the killing of a female's young must result in her ovulating and mating with the infanticidal male sooner than she could have had her young not been killed (ovulation is inhibited in lactating female mice); and (3) infanticide must be mediated, at least in part, genetically and thus be heritable.

This chapter describes laboratory experiments with house mice (*Mus musculus*) designed to test the first two predictions of the sexual selection hypothesis. The third prediction, that concerning the relationship between genotype and the tendency for male mice to commit infanticide, is addressed by another chapter in this volume (see Chapter 20 by Svare *et al.*), although the present work clearly demonstrates that within a given strain of mice, sex hormones regulate both the prenatal development and adult expression of infanticide. Thus, the problems associated with examining the genetic basis of a behavior that is modulated both by hormones and by experience are also mentioned in this chapter.

METHODS

The test procedure utilized in most of the experiments consisted of placing two 1-day-old mice into the cage of a male and then examining the pups 30 min later. One of three possible behavioral responses was recorded at this time: (1) infanticide—one or both pups were dead; (2) parental behavior—one or both pups were in a nest with the male hovering over them, and the pups were warm; or (3) ignore—neither pup was in the nest or wounded, and both pups were cold (newborn mice cannot thermoregulate). The length of time that the young are left with the male does not appear to be important (15 min to 4 hr; Svare and Broida, 1982; F. vom Saal: unpublished observation). Either infanticide or parental behavior occur almost immediately after young are introduced into a male's cage. Neither the age (newborn, 7 days old, 10 days old) nor the sex of the young influences the tendency of males to exhibit infanticide or parental behavior (Svare and Broida, 1982; vom Saal and Howard, 1982; F. vom Saal: unpublished observation).

Other testing procedures have also been utilized. For example, in some experiments, males were allowed to enter chambers containing females and their nursing young. But, moving males from one cage to another is stressful, and this could potentially interact with other variables in influencing the behavior of male mice toward young. The procedure of placing the young into a male's cage was thus utilized in most experiments so that the greatest number of variables were controlled.

This procedure not only involves the least amount of disturbance to the male but results in a minimal loss of life of young relative to procedures involving the placement of a male with a female and her entire litter.

All males utilized in these experiments were housed individually when 35 days old. Between Day 35 and 40 after birth, blood concentrations of testosterone begin to increase dramatically in male mice, and if males remain housed in groups after this age, they begin to fight (McKinney and Desjardins, 1973a). As will become clear, control of fighting experience is necessary in studies of infanticide in mice.

Unless otherwise indicated, testing for infanticide occurred in early adulthood between 75 and 90 days of age. The tendency of male mice to commit infanticide changes during adolescence, and the ontogeny of infanticide varies in different strains of mice. For example, fewer CF-1 male mice committed infanticide when tested between 55 and 60 days of age ($n = 204$; infanticide = 29%, parental = 55%, ignore = 16%) than when tested again at 90 days of age ($n = 204$; infanticide = 49%, parental = 46%, ignore = 5%; F. vom Saal, in press). In C57BL/6J mice, no change in the frequency of infanticide is observed after males reach 55 days of age (70% commit infanticide), while in DBA/2J male mice, a dramatic increase in the frequency of infanticide occurs between 65 days of age (20% commit infanticide) and 135 days of age (65% commit infanticide; Svare and Mann, 1981).

Statistical Analyses

An important issue in studies of infanticide is the unit of analysis that should be used. In the studies discussed here, individual males serve as the units of analysis, since it is the behavior of particular individuals that is being examined. While this convention may seem obvious, previous studies (cf. Mallory and Brooks, 1978; Labov, 1980; Huck, Soltis, and Coopersmith, 1982) have utilized individual young within a litter as the unit of analysis. Quite often, these previous studies have provided no information concerning how many male mice actually committed infanticide in any experimental condition, but instead have just reported the aggregate number of young killed by all males tested in that condition. This methodological problem has been compounded by statistical testing of such pup mortality data using Chi-square analysis. The use of the Chi-square test on pup mortality data violates a basic assumption of this analytical method, namely, that the data points be statistically independent of each other. Since this latter requirement is fulfilled only when males, not pups, are used as the unit of analysis, the conclusions of many previous studies of rodent infanticide are considered open to question.

MATING-INDUCED, TIME-DEPENDENT FACILITATION AND INHIBITION OF INFANTICIDE

The experiments described in this section were undertaken following the observation that male mice used as colony studs were significantly less likely to commit infanticide than were sexually naïve males. This prompted the question whether mating experience had influenced infanticide in male mice. The data presented in Table I reveal the remarkable effect that mating experience has on the tendency of male CF-1 mice to either commit infanticide or behave parentally toward young. In this experiment, 240 male CF-1 mice were paired with females for 3 hr, after which time the females were examined for vaginal plugs and removed from the male's cage (Day 0 = the day of mating). The males were then randomly assigned to groups to be tested for infanticide at different intervals after mating (30/group). The behavior of these groups of males toward young was compared with the behavior of a group of 30 sexually naïve males (about 45 to 50% of naïve, adult CF-1 male mice commit infanticide). Four days after mating, there was a significant increase in the proportion of males committing infanticide (83%) compared to the naïve controls (50%). However, 12 days after mating, just the opposite was the case: There was a significant decrease in the proportion of males that committed infanticide (10%) compared to controls and an increase in the proportion of males that behaved parentally (83%) compared to controls (23%). This inhibition of infanticide was also observed in males tested at 20, 35, 42, and 50 days after mating. Between 50 and 60 days after mating, however, there was again a facilitation of infanticide and inhibition of parental behavior similar to that observed 4 days after mating. Males tested at both

Table I. Effect of mating on infanticide^a

Behavior	Pre-mating	Days after mating							
		4	12	20	35	42	50	60	90
Infanticide	50	83	10	10	17	30	23	77	73
Parental	23	17	83	80	63	63	57	17	23
Ignore	27	0	7	10	20	7	20	6	4
χ^2 versus pre-mating group:		10.8	21.3	19.6	10.8	10.6	7.4	5.6	6.8
Significance level (<i>p</i>):		<0.005	<0.001	<0.001	<0.005	<0.005	<0.05	<0.05	<0.05

^a The percentage of different groups of male mice (30 males/group) that committed infanticide, behaved parentally toward, or ignored two newborn mice that were placed into a male's cage for 30 min. The males were tested at different times after mating with a female.

60 and 90 days after mating were significantly more likely to commit infanticide than were controls (F. vom Saal, in press).

Another 90 males were tested for infanticide, allowed to mate with a female, and then retested for their behavior toward young 1, 8, or 20 days after mating (30/group). The results presented in Table II reveal that infanticide is enhanced relative to pre-mating levels by 1 day after mating. However, males tested 8 days after mating behaved as if they had not mated at all: Only 1 out of 30 males did not exhibit the same behavior on both the pre- and postmating tests. Finally, most of the males tested 20 days after mating behaved parentally. Chi-square analysis performed on the postmating data confirmed that there was a significant effect of length of time since mating on the behavior of males toward young.

The males that were tested with newborn young 20 days after mating (see Table I) were also tested with 10-day-old young 35 days after mating. The objective was to determine whether the behavior of males would be different toward newborn young and toward young that were near the actual age that the male's most recent mating would have produced (parturition is 19 days after mating.) When tested with 10-

Table II. Effect of mating pretest/retest on infanticide^a

Behavior	Pretest	Retest after mating
<i>One day</i>		
Infanticide	47	83
Parental	33	13
Ignore	20	4
<i>8 days</i>		
Infanticide	47	50
Parental	43	43
Ignore	10	7
<i>20 days</i>		
Infanticide	43	23
Parental	43	77
Ignore	14	0

^a Percentage of males that committed infanticide, were parental toward, or ignored two newborn mice that were placed into the male's cage for 30 min. All males were administered a pretest and then allowed to mate with a female 1 week later. The males were then randomly assigned to three groups (30 males/group) and retested at different intervals after mating, 1 versus 8 versus 20 days after mating: $X^2(4) = 25.9$, $p < 0.001$.

day-old mice, only one of the males did not exhibit the same behavior shown on Day 20. Thus, male mice do not behave differently toward newborn and 10-day-old young when tested on Day 35 after mating.

Mice that are between 14 and 20 days old are very active; the eyes open at 13 days of age, and they have a coat of fur by this time. When tested with juvenile mice that were between 14 and 20 days old, most males that had mated between 3 and 6 weeks prior to testing still behaved parentally. It appears, however, that some degree of conflict is engendered in the males by 14–20-day-old young, since many males appeared highly agitated and often roughly groomed the young (F. vom Saal, in press).

As a control for the possibility that males may not reliably exhibit the same behavior toward young on repeated tests, 150 sexually naïve males were tested for infanticide and then retested at 4, 8, 14, 35, or 60 days after the first test (30/group). With few exceptions, males that committed infanticide on the first test committed infanticide on the second test (F. vom Saal, in press).

Pregnancy in house mice lasts about 3 weeks (19–21 days). Young are typically weaned about 4 weeks after birth and, depending on population density, disperse from the natal territory at puberty about 4 weeks after birth (Lidicker, 1975; Bronson, 1979). A male mouse would thus typically be in contact with its own preweanling young between 3 and 7 weeks after mating. The finding that between 2 and 7 weeks after mating there is a significant inhibition of infanticide in male mice is thus striking. In essence, males tend not to be infanticidal at those times that they would be likely to come in contact with preweaning young that they had sired. Within less than 4 days or beyond more than 60 days postmating, there is actually a facilitation of infanticide in previously mated male mice compared to sexually naïve males. Taken together, these findings certainly appear consistent with the hypothesis that selection has operated to inhibit infanticide during the time that a male mouse might kill its own offspring. In addition, infanticide is facilitated at times when a male would encounter the nursing young of another male.

Effects of Mating Experience on Parental Behavior

Mating not only influences infanticide in male mice but also parental behavior. Between 2 and 7 weeks after mating, most male mice build a maternal-like nest, retrieve the young, stimulate micturition in the young by licking the genitals periodically, and hover over the young to maintain their body temperature. These observations suggest that selection has operated not only to reduce infanticide but also to increase parenting behaviors, again, specifically during those times that

male mice would be in contact with their own preweanling young. It is difficult to imagine that the facilitation of parental behavior in male mice between 2 and 7 weeks after mating could be so precisely coupled with the length of pregnancy (19 days) and lactation (25–30 days) in female mice unless parenting behaviors in males were also acted on by natural selection (i.e., were adaptive). It could be argued that perhaps these individual parenting behaviors are merely on the opposite end of a behavioral continuum from infanticide such that as the incidence of infanticide decreases, the incidence of this complex of parenting behaviors automatically increases. However, additional evidence (see following) suggests that infanticide and parental behavior are, in fact, independent processes.

Evidence that the characteristics of the young influence the maintenance of parental behavior once it is initiated in male mice was obtained by repeatedly exposing the same 30 males that had been tested for infanticide 4 days after mating (see Table I, Day 4 Group) to newborn young 12, 20, 35, 50, 60, 70, 80, and 90 days after mating. The results of this longitudinal design are presented in Table III and reveal that between the fourth and twelfth day following mating, there was a complete reversal of behavior. Infanticide was facilitated and parental behavior was inhibited on Day 4, while on Day 12 infanticide was inhibited and parental behavior was facilitated. The repeated exposure to young served to maintain parental behavior 90 days after mating (73% of the males behaved parentally; see Table III, Day 90), while most of the males tested for the first time 90 days after mating committed infanticide (73% of these males committed infanticide; see Table I, Day 90 Group). This difference is statistically significant ($\chi^2(2) = 22.5$, $p < 0.001$; F. vom Saal, in press). When the litters of postpartum female rats (Bruce, 1961a; Rosenblatt, Siegel, and Mayer, 1979) or mice (Svare, 1981) are replaced daily with newborn young, behavior characteristic of a female that has just delivered a litter is maintained for extended periods of time (several months). Thus, the young serve as stimuli for eliciting parental behavior in adult females, and as the characteristics of the young change, the behavior of the adult also changes.

Effects of Repeated Mating

Consider the situation in which a male mated with one female and then between 2 and 3 weeks later mated with a second female. It would clearly not be advantageous for the second mating to facilitate infanticidal behavior, since the male may well come into contact with young produced by his first mating. To examine this situation, 14 naïve males were tested for their behavior toward young (43% committed infanticide, 50% behaved parentally, and 7% ignored the young). These

Table III. Effect of repeated exposure to young on infanticidal behavior^a

Behavior	Days after mating								
	4	12	20	35	50	60	70	80	90
Infanticide	83	10	0	13	13	17	20	20	24
Parental	17	87	93	77	80	73	77	77	73
Ignore	0	3	7	10	7	10	3	3	3

^a The percentage of a single group of 30 mice that committed infanticide, behaved parentally toward, or ignored two newborn mice that were placed into a male's cage for 30 min. These males were first tested 4 days after mating with a female (see Table I, Day 4 Group) and then were retested at 8-15-day intervals.

males were then mated with first one female (Day 0) and then with a second female 15 days later. Four days after the second mating (19 days after the first mating), infanticide proved to be inhibited and parental behavior facilitated (14% of the males committed infanticide, and 86% behaved parentally; F. vom Saal, in press). Thus, the increase in the incidence of infanticide observed in sexually naïve male mice 4 days after mating was not observed in males that had first mated 19 days and then again 4 days prior to coming in contact with young. Apparently, the facilitation of infanticide within 4 days of mating only operates in sexually naïve males.

There is little precedent in the literature for the time-dependent changes that occur following mating in the behavior of male mice toward young. Particularly unusual is the cyclical facilitation, inhibition, and then facilitation of a behavior as a function of time. However, once male mice become parental, the repeated opportunity to behave parentally can serve to disrupt this temporal sequence and to sustain an inhibition of infanticidal behavior.

Mechanism of Facilitation and Inhibition

At this time, three experiments that were designed to investigate the particular aspect of mating that produces the observed changes in male behavior have been completed. First, a group of 30 male mice were placed with 2 diestrous, nonreceptive females for 24 hr. The absence of mating was verified by repeated examination of the females. Three weeks later, the males were tested with 2 newborn young, and 53% of the males committed infanticide, 27% behaved parentally, and 20% ignored the young. Cohabiting with a female but not mating thus has no effect on either infanticide or parental behavior in male mice. Second, 30 males were pretested for their behavior toward young (56% committed infanticide, and 44% behaved parentally). Two weeks later, the males were allowed to mount (at least 20 times with intromissions)

a sexually receptive female, but the females were removed before the males ejaculated. The males were retested 1 day and 20 days later for their behavior toward young. Virtually no change in behavior relative to the pretest occurred in these males at either 1 day (56% were infanticidal) or 20 days (40% were infanticidal). Third, 30 males were pretested for their behavior toward young (43% were infanticidal). Two weeks later, the males were allowed to ejaculate when paired with a sexually receptive female. As soon as the males ejaculated, they were housed individually in a clean cage, thus preventing any post-ejaculatory contact with the female. One day after ejaculating, 83% of these males committed infanticide, while 20 days after ejaculating, 3% committed infanticide (F. vom Saal, in press).

In sum, males that mount and intromit, but do not ejaculate, when paired with a female do not change their behavior toward young, while males that ejaculate but have no postejaculatory contact with a female do change their behavior toward young. It is thus proposed that some aspect of ejaculating with a female (such as vaginal stimulation of the engorged penile cup that forms during ejaculation: McGill and Coughlin, 1970) may serve as the "trigger" for the facilitation and then inhibition of infanticide following mating in male mice. It is interesting that penile engorgement at ejaculation has a significant effect on sperm motility within the uterus and on the establishment and maintenance of pregnancy (McGill, 1970; McGill and Coughlin, 1970; Adler, 1983; Adler, Allen, and Toner, 1982). Male mice have spontaneous ejaculations almost every night (Huber and Bronson, 1980), but these ejaculations (which may be physiologically different from ejaculations during coitus) obviously do not lead to the changes in behavior toward young that occur after ejaculating during coitus in male mice.

INFANTICIDE IN F_1 WILD MALE AND FEMALE MICE

In laboratory mice, there is a pronounced sex difference in the incidence of infanticide, with less than 10% of females typically committing infanticide (Svare *et al.*, Chapter 20, this volume). But, in one recent study using a testing procedure very similar to that described previously, nearly all mice, both male and female, committed infanticide in the F_1 - F_2 generation descended from wild house mice trapped in Israel (Jakubowski and Terkel, 1982). In contrast, the F_1 generation descended from wild house mice trapped in a field near Columbia, Missouri did exhibit a sex difference in infanticide. Specifically, 60 males and 82 females were tested for their behavior toward young at 75 days of age. The animals were housed between 2 and 4 per cage from weaning until 5 days before behavior testing, at which time all animals were individually housed. Males and females differed signifi-

cantly in their behavior toward one newborn wild-mouse pup that was placed into a corner of the cage. Of the males, 87% were infanticidal compared to 61% of the females.

Sexually naive wild male mice were also placed into cages containing a wild female mouse and her 2-day-old young, and 15 of 16 males committed infanticide. Other wild males were paired with wild females for 14 days, and then both the male and the female were rehoused individually in clean cages. Two days after the prior female partner had delivered, the males were placed: (1) with their prior partners ($n = 10$; 0% were infanticidal) or (2) with a novel female that had also delivered 2 days previously ($n = 20$; 5% were infanticidal). All animals were observed for 30 min after pairing and then examined daily. The same experiment was conducted with CF-1 mice (11 males/group), and none of the males committed infanticide. Infanticide is thus also inhibited in wild mice 3 weeks after mating, and recognition of the prior female partner is not a factor in this inhibition in either wild or CF-1 mice (M. McCarthy and F. vom Saal, unpublished observation).

F₁ female wild mice have also been examined for their behavior toward young during pregnancy. Females that had committed infanticide prior to becoming pregnant are still infanticidal 2-12 hr prior to delivery. In addition, many females that had previously ignored or parented young prior to mating committed infanticide when tested just prior to parturition. When 22 virgin females were tested prior to mating, 63% committed infanticide, while 91% of these same females committed infanticide just prior to parturition. But, when these females subsequently delivered their own young, they all behaved parentally, suggesting that some variable associated with parturition (such as vaginal-cervical stimulation) served to inhibit infanticide and induce the onset of parental behavior (M. McCarthy and F. vom Saal: unpublished observation). Vaginal-cervical stimulation has been reported to influence the onset of parental behavior in sheep (Keverne *et al.*, 1983).

INTERACTION OF DOMINANCE STATUS AND PRIOR MATING EXPERIENCE

Where stable territories are observed in populations of house mice, a deme (breeding group) generally consists of between 2 and 4 males and 6 and 8 females (Bronson, 1979). An interesting feature of mouse demes is a nonlinear dominance hierarchy among males and possibly also among females (Lloyd and Christian, 1969; Christian, 1971; vom Saal, 1981). Thus, there is only one dominant male within a deme, and all other males are subordinate. How likely these subordinate males are to mate and produce young is a matter of considerable debate (cf. Oakeshott, 1974), but there is general agreement that dominance status does influence reproductive success in male and female mice

(cf. Lloyd and Christian, 1969). For example, in one study utilizing genetic markers, over 90% of offspring were reported to be produced by the one dominant male in a breeding group (DeFries and McLearn, 1972). Given that excess subordinate males remain within a mouse deme and that most wild male mice are infanticidal, the obvious question arises as to whether subordinate males might kill the offspring of the dominant male.

The influence of dominance status on infanticide was examined in adult male CF-1 mice. Males were paired for 1 hr each day for 7 days, and dominance status was assessed by observations of fighting and rates of urine marking. Males of known dominance status were then tested for their behavior toward newborn young. Table IV shows that relative to males that had neither mated nor fought (see Table I, pre-mating group), the achievement of dominance-enhanced infanticide in males that had never mated (Table IV, C). Conversely, defeat in fighting and subordinate status resulted in an inhibition of infanticide (Table IV, A). This same experiment was next repeated with males that had mated 2 weeks prior to their fighting experience; testing for infanticide thus occurred a total of 22 days after mating. Prior mating experience eliminated the facilitation of infanticide associated with achieving dom-

Table IV. Influence of dominance status on infanticidal behavior^{a,b}

Behavior	Subordinate		Dominant		Retest of Group D: 90 days after mating (n = 33)
	(A) Sexually naïve (n = 22)	(B) 22 Days after mating (n = 25)	(C) Sexually naïve (n = 34)	(D) 22 Days after mating (n = 33)	
Infanticide	23	28	82	15	88
Parental	41	60	12	76	12
Ignore	36	12	6	9	0

^a Percentage of adult male mice that either killed, were parental toward, or ignored two newborn mice that were placed into the male's cage for 30 min. A group of males were allowed to mate with two females while another group of males remained sexually naïve. Fourteen days later, males within each of these groups were paired for 7 days (1 hr per day) and categorized as being dominant or subordinate. All dominant and subordinate males that were sexually naïve as well as dominant and subordinate males that had previously mated were then tested for their behavior toward young. The dominant males that had previously mated (Group D) were retested 10 weeks later (90 days after having mated) (vom Saal and Howard, 1982).

^b Group A versus C: $\chi^2(2) = 19.9$, $p < 0.001$. Group C versus D: $\chi^2(2) = 31.4$, $p < 0.001$. Group A versus B: not significant. Group B versus D: not significant.

inance in male mice: Parental behavior was facilitated and infanticide inhibited in dominant males 22 days after mating, similar to the effect of mating on the behavior of males that had not previously fought with another adult male (see Table I, Day 20 Group). In contrast to the foregoing result, prior mating experience did not have a significant effect on infanticide in subordinate males (Table IV, B), although there was a tendency for more of the previously mated subordinate males to exhibit parental behavior than was the case for the sexually naïve subordinate males (vom Saal and Howard, 1982).

Dominant males that were tested for infanticide 22 days after they had mated (see foregoing) were retested for infanticide 90 days after they had mated (10 weeks after the first test). At this time, 88% of the males committed infanticide compared to 15% during the previous test 22 days after mating (see Table IV; vom Saal and Howard, 1982). This finding again confirms the previous observation (Table I, Day 90 Group) that 90 days after mating most males commit infanticide.

The results of the previous experiments demonstrate that the tendency of a male mouse to commit infanticide is strongly affected by dominance status. It has been proposed (vom Saal and Howard, 1982) that as a result of continuous defeat, subordinate males within a deme are inhibited from committing infanticide. But, if the dominant male within a deme dies, the subordinate males will fight, and one of them will become the dominant male (cf. Reimer and Petras, 1967; Lidicker, 1976). Since achievement of dominance appears to facilitate infanticide in male mice that have not previously mated, it was proposed that a new dominant male would most likely kill the offspring of his predecessor (an assumption of this hypothesis is that subordinate males do not mate). Although this action at first glance would appear to clearly be advantageous for the new dominant male, it is important to point out that subordinate males in mouse demes are most likely brothers, half-brothers, or offspring of the dominant male. The new dominant male may thus be related by a factor of from 12.5 to 25% on average to the dominant male's offspring that it was killing.

INFANTICIDE AND REPRODUCTIVE SUCCESS IN MALE MICE

The previous experiments reveal that newly dominant male mice kill the young of other males. Infanticide in such a situation is proposed to be adaptive. The potential increase in fitness associated with infanticide by a newly dominant male depends on the relatedness of the male to the victims of the attacks and on whether committing infanticide increases the rate of production of young by an infanticidal male. To examine this latter question, 40 sexually naïve male mice were each

placed into one side of a chamber divided by a wooden partition; the other side of each chamber was occupied by a female that had delivered a litter (culled to 8 young) within the previous 15 hr. After 30 min, the partition was removed and the males allowed to cohabit with the females. Upon removal of the partition, 22 of the males immediately killed all of the young, while in 10 cases all of the young survived to weaning. In 8 cases, up to 3 of the 8 young survived to weaning. As predicted by the sexual selection hypothesis, the number of days to the delivery of the subsequent litter (sired by the introduced male) was found to vary significantly as a function of whether or not the introduced male committed infanticide. For females paired with an infanticidal male, the mean (\pm SE) interval was 22.1 ± 0.9 days compared to 29.8 ± 1.9 days for females paired with a noninfanticidal male (*t*-test, $p < 0.001$). The reason why only a few young survived in 8 of the litters is unknown, but the mean length of time for the next litter to be born under these conditions was 25.5 ± 2.8 days. None of the 40 males killed any of the young in the litter that they had sired (vom Saal and Howard, 1982).

The timing of male introduction in relation to parturition is an important feature of the preceding experiment. The first night after a litter is born, female mice go through a period of postpartum estrus. Implantation of the embryos is delayed in female mice that mate at postpartum estrus and subsequently lactate. The length of the delay varies in different mouse strains and also depends on the number of nursing young: the larger the litter, the longer the delay (Mantelenakis and Ketchell, 1966). In lactating CF-1 mice, the delay in implantation following insemination at postpartum estrus is 1 week (F. vom Saal: unpublished observation), which is exactly the difference in the length of time to produce the next litter observed in the previous experiment for the infanticidal and noninfanticidal males. This finding suggests that the difference between infanticidal and noninfanticidal males in the length of time to produce young is due to the fact that implantation was delayed in the females that continued to nurse young after insemination during postpartum estrus. Thus, males were placed with females prior to postpartum estrus to provide the strongest test of the hypothesis that committing infanticide would significantly shorten the interval males must wait prior to the production of their own offspring. If males had been placed with lactating females on the day after postpartum estrus, noninfanticidal individuals inevitably would have experienced a delay of at least 1 month before the original litter was weaned and the female ovulated again.

The same experiment was repeated with 20 males that had mated with another female 3 weeks prior to being placed into boxes containing

a lactating female (the females had each been inseminated by another male). Upon removal of the partition, the males were observed with the female and her litter for 30 min, and during this time none of the males was observed killing young. The males again remained with the females until 5 days after their own offspring were born. Under these circumstances, the interval to the birth of the subsequent litter that was sired by these males was 28.3 ± 2.0 days. Of the 160 young that were alive at the start of this experiment (8 young/female), only a total of 6 young did not survive to weaning at 23 days old, and no more than 2 young died in any litter. This percentage of young is lost even when males are not present, suggesting that these few deaths were attributable to natural causes and not infanticide (vom Saal and Howard, 1982).

Not only do these studies show that infanticide can be reproductively advantageous for male mice in some circumstances, but they also confirm, using a different testing procedure, that prior mating experience completely inhibits infanticide in male mice 3 weeks after mating. In CF-1 male mice, (as well as wild males), recognition of either the individual female with which a male mated or of genetically related young does not appear to play any critical role in modulating infanticide and parental behavior. In contrast, there is a recent report that in Swiss Webster mice, recognition by males of the females with which they have mated plays a role in the inhibition of infanticide (Huck *et al.*, 1982).

FEMALE COUNTERSTRATEGIES TO INFANTICIDE

Female mice do not exhibit intense aggression toward intruders into the nest area until after postpartum estrus (Svare, 1981). Perhaps this is an unavoidable consequence of the fact that a female mouse cannot simultaneously mate with and attack a potentially infanticidal male during postpartum estrus. Nevertheless, during the first 2 days after birth, newborn mice may be particularly vulnerable to infanticide by a new dominant male in a deme. One possible female counterstrategy to infanticide in this situation may involve communal nesting. Females with different aged young may share and jointly defend a common nest area. Since female mice in a deme are probably related, protection of another female's young should result in an increase in inclusive fitness. Precisely this sort of communal nesting in a freely growing population of CF-1 mice has been observed (F. vom Saal: unpublished observation). Investigators working with other laboratory and wild strains of mice (Saylor and Salmon, 1971; Lloyd, 1975) have made similar observations. These observations are far from conclusive, and it is clear that the issue of possible female counterstrategies to infanticide (such as communal nesting) needs to be examined in a study of wild mice in a natural or seminatural environment (see Schwagmeyer, 1979,

and Huck, Chapter 18, this volume, for a more detailed discussion of this issue).

FOETAL HORMONES AND INFANTICIDE: THE INTRAUTERINE POSITION PHENOMENON (IUP)

Of the three predictions of the sexual selection hypothesis cited at the beginning of this chapter, only one remains to be substantiated, namely, that the tendency of males to commit infanticide (and also to behave parentally) is at least partially genetically determined and thus heritable. Presumably, once the complex of genes involved in regulating infanticide appears in a population of mice, there would rapidly be an increase in the frequency of this complex in the population (Hausfater *et al.*, 1982b), and this would probably be true even in situations in which the selection coefficient was low (Wills, 1981). However, the fact that infanticide is modulated (along with aggression, sex behavior, urine marking, and other behaviors; see reviews by Beatty, 1979; Gorski, 1979; vom Saal, 1983b) by hormones during both perinatal and adult life in mice makes the study of the genetic basis of infanticide quite complicated. Traits that are modulated hormonally, and which are also influenced by experience, are thought to be polygenic, and it has proved extremely difficult to develop either mathematical models or analytical techniques for determining with any precision the genetic contribution to such behaviors (cf. Lande, 1980).

A random developmental event in polytocous (multiple-birth) species, including mice, is the position that a foetus occupies in the uterus relative to foetuses of the opposite sex. The intrauterine position phenomenon (IUP) refers to the fact that foetuses can develop next to, and possibly be influenced by the hormonal secretions of, foetuses of the same or opposite sex (Fig. 1; vom Saal, 1983d; vom Saal and Bronson, 1980a; vom Saal, Grant, McMullen and Laves, 1983). Since intrauterine position is a random event, male foetuses that develop next to female foetuses do not differ systematically in genotype from male foetuses that develop next to other male foetuses (vom Saal, 1981). This phenomenon is thus of particular relevance to the problem of determining what component of the variance in the tendency of male mice to commit infanticide is mediated genetically (vom Saal, 1983a). To obtain offspring from known intrauterine positions, female mice are time-mated, and the young are delivered by Cesarean section and raised by foster mothers (vom Saal, 1981).

Hormonal Consequences of IUP

During the period of sexual differentiation (in mice between about Day 14 of gestation through the first week of postnatal life), males secrete higher titers of testosterone than do females (vom Saal and

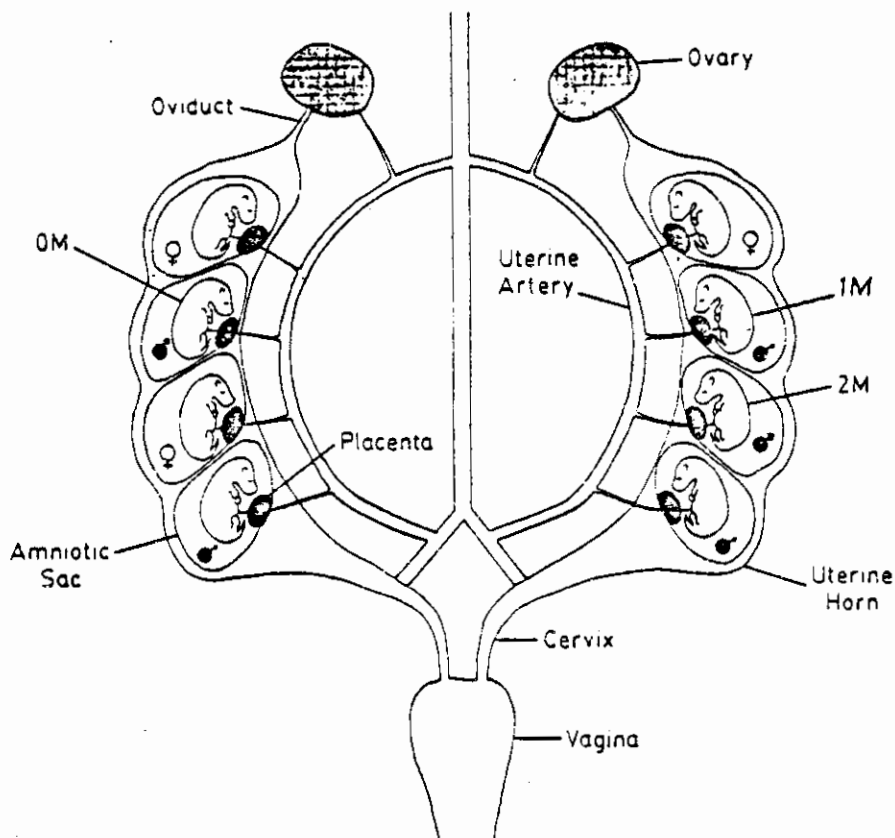


Figure 1. Schematic diagram of the uterine horns and uterine loop arteries of a pregnant mouse at term. The intrauterine position of fetuses is determined at Cesarean delivery. The labels OM, 1M, and 2M refer to the number of male fetuses that an individual is contiguous to (2M = between 2 males, 1M = next to 1 male, and OM = between 2 females). This scheme is used to identify both male and female fetuses, but only males are labeled in this figure.

Bronson, 1980a; Weisz and Ward, 1980). During prenatal but not postnatal life, female mouse fetuses that are located between two male fetuses (2M females) have higher amniotic fluid and blood titers of testosterone than do female fetuses that do not develop next to a male fetus (OM females; vom Saal and Bronson, 1980a), and intrauterine position thereby influences morphology, physiology, and behavior in female mice (vom Saal and Bronson, 1978; 1980b; vom Saal, Pryor and Bronson, 1981). These findings led to the prediction that male mice that developed between two male fetuses (2M males) may also differ in prenatal hormone titers and in adult behavior from males that developed in utero between two female fetuses (OM males). Contrary to

this expectation, 0M and 2M male mouse fetuses were not found to differ in their blood or amniotic fluid titers of testosterone. But, female mouse fetuses were found to have significantly higher amniotic fluid titers of estradiol than male fetuses, and 0M male fetuses had significantly higher amniotic fluid titers of estradiol than did 2M male fetuses (vom Saal *et al.*, 1983). Estradiol in the amniotic fluid is presumed to be in equilibrium with estradiol in the foetal bloodstream (Belisle and Tulchinsky, 1980).

IUP and the Activational Effects of Testosterone

Prior research had revealed that infanticidal tendencies in mice were influenced by hormones during both prenatal and early postnatal life (Gandelman and vom Saal, 1977; Svare *et al.*, Chapter 20 this volume). 0M and 2M male mice, as well as 1M males (those that developed between a male and a female foetus in utero), were therefore compared for their behavior toward two newborn mice that were placed into the home cage of each male as described previously. The results presented in Table V reveal that intrauterine position strongly influenced the behavior of male mice toward young. Most 0M males committed infanticide and most 2M males behaved parentally; 1M males were equally likely to kill or behave parentally toward young (vom Saal, 1983c).

There is evidence that indicates that parenting behaviors and infanticide are not influenced by hormones in the same way during perinatal life in male mice. Parenting behaviors and infanticide thus appear to be independent and not simply responses to young that represent oppo-

Table V. Effect of intrauterine position on infanticide^a

Behavior	Intrauterine position of male ^b			Total
	2M	1M	0M	
Infanticide	23	40	63	42
Parental	67	37	27	43
Ignore	10	23	10	15

^a The percentage of gonadally intact, 75-day-old 0M, 1M, and 2M male mice (30/group) that committed infanticide, were parental toward, or ignored two newborn mice that were placed into a male's cage for 30 min (vom Saal, 1983c).

^b $\chi^2(4) = 14.2$ $p < 0.01$. 2M = between two male fetuses; 1M = between a male and female foetus; 0M = between two female fetuses.

site ends of a behavioral continuum. The evidence supporting this hypothesis was obtained in a study utilizing 0M and 2M male mice that had been castrated at birth or at 28 days of age (about 1 week prior to puberty). These males were tested for their behavior toward young when they were 75 days old without being treated with exogenous hormones. The results presented in Table VI reveal that most of the 0M males and the 2M males that had been castrated at birth behaved parentally or ignored the young. In contrast, significantly more 2M males than 0M males behaved parentally when castrated at 28 days of age. Thus, more 2M males behaved parentally toward young regardless of whether castration had occurred at 28 days of age (Table VI[B]) or whether they were gonadally intact (Table V).

A possible explanation for these findings is that at birth, most males (regardless of prior intrauterine position) have the potential to behave parentally, but the tendency to behave parentally is suppressed some time between birth and 28 days of age in gonadally intact 0M but not 2M males. In other words, it is suggested that males that developed in utero between male fetuses are influenced by the hormonal secretions of their testes after birth in a different way from those males that developed in utero between female fetuses. Since the difference between 0M and 2M males in the tendency to commit infanticide required the presence of testes or exogenous testosterone to be observed, while the difference between 0M and 2M males in the tendency to behave parentally toward young did not require that the animals be gonadally intact or treated with testosterone, it is concluded that the method by which infanticide and parental behavior are mediated by hormones is quite different.

Table VI. Effect of pre- and postnatal hormones on infanticide^a

Behavior	(A) Castrated at birth ^b		(B) Castrated when 28 days old ^c	
	2M (n = 20)	0M (n = 20)	2M (n = 18)	0M (n = 14)
Infanticide	15	10	0	14
Parental	60	50	61	14
Ignore	35	40	39	72

^a The percentage of adult 0M and 2M male mice that had been castrated at Cesarean delivery or when 28 days old that committed infanticide, were parental toward, or ignored two newborn mice that were placed into the male's cage for 30 min. The males were tested without being treated with testosterone when they were 75 days old (vom Saal, 1983c).

^b Not significant.

^c Parental versus nonparental: $\chi^2(1) = 5.4$. $p < 0.05$.

These findings are particularly intriguing since the influence of perinatal testosterone exposure on infanticide is opposite to that for aggression between adult males (vom Saal, 1983b). Exposure of mice to elevated titers of testosterone during early life results in an increase in sensitivity to the activational effects of testosterone on intermale aggression in adulthood (vom Saal, 1979). In contrast, for infanticide, a mouse that is exposed to elevated titers of testosterone around the time of birth is less sensitive to the activational effects of testosterone on infanticide in adulthood (i.e., less likely to commit infanticide) than is a mouse that is exposed to low titers of testosterone during early life (Gandelman and vom Saal, 1977; Samuels *et al.*, 1981). Thus, both intermale aggression and infanticide are behaviors that are modulated by testosterone during early life and then activated by testosterone in adulthood, but during perinatal life testosterone appears to have a sensitizing effect on the neural areas mediating intermale aggression and a desensitizing effect on the neural areas mediating infanticide in terms of the capacity of these neural areas to respond to testosterone in adulthood. It may seem contradictory that circulating testosterone should be necessary for both intermale aggression and infanticide to be exhibited by naïve, adult, male mice, while the same hormone could have such different effects on these behaviors during an earlier period in life. During development, however, enzyme systems as well as hormone receptors, which allow tissues to respond to hormones, are known to change in some tissues. Thus, for the neural tissues mediating infanticide, testosterone appears to be inhibitory during early life and then, at some point prior to adulthood, there is a transition, and testosterone activates these same neural areas. It is also interesting that intermale aggression is activated by testosterone during the early pubertal period (around 35 to 40 days of age), while a dramatic increase in the proportion of CF-1 male mice that commit infanticide is observed after 60 days of age (see foregoing). Taken together, the findings lead to the prediction that male mice that were the most sensitive to the activational effects of testosterone on intermale aggression would be the least likely to commit infanticide.

To examine the hypothesis that adult 0M and 2M males would differ in their sensitivity to both the infanticide- and aggression-inducing action of testosterone, different groups of 0M and 2M male mice were castrated at Cesarean delivery and then in adulthood were administered testosterone contained in a silastic capsule. These males were subsequently tested either for aggression against another adult male or for their behavior toward two newborn mice. In the aggression experiment (see Table VII[B]), more 2M than 0M males exhibited a 5-sec sustained attack toward a male opponent within the 16-day test period (vom Saal *et al.*, 1983). In the infanticide study, more 0M than 2M

Table VII. Effect of testosterone treatment on aggressive behavior

Behavior	(A) Behavior toward young ^a	
	2M	0M
Infanticide	50	78
Parental	39	15
Ignore	11	7

Behavior	(B) Intermale aggression ^b	
	2M	0M
Percentage attacking within 16 days	70	40

^a The percentage of 100-day-old 0M and 2M male mice (28/group) that committed infanticide, behaved parentally toward, or ignored two newborn mice that were placed into a male's cage for 30 min. The males were castrated at birth and, in adulthood, administered testosterone (contained in a silastic capsule) for 25 days (vom Saal, 1983c). $\chi^2(2) = 3.4$; $p = 0.18$.

^b The percentage of 0M and 2M male mice (20/group) that attacked a male intruder. The males were castrated at birth. When the males were 75 days old, 10-minute tests for aggression were administered every other day for 16 days after the males were implanted subcutaneously with a silastic capsule containing testosterone (vom Saal *et al.*, 1983). $\chi^2(1) = 3.6$; $p < 0.05$.

males committed infanticide, while more 2M than 0M males behaved parentally after 25 days of testosterone treatment, although this difference was not statistically significant (Table VII[A]; vom Saal, 1983c). Thus, as predicted, one consequence for male mouse fetuses of developing between two female fetuses is an enhanced sensitivity to the activational effects of testosterone on infanticide and decreased sensi-

tivity to the activational effects of testosterone on aggressiveness toward other males.

The Hormonal Basis of Differences in Behavior due to Intrauterine Position

The data indicate that 0M male mice do not differ from 2M males in their blood testosterone levels during foetal life, but 0M males do have higher titers of estradiol. This finding led to the hypothesis that during foetal life, circulating estradiol attenuates the organizational effects of testosterone on the neural tissues mediating intermale aggression and infanticide (vom Saal, 1983d; vom Saal *et al.*, 1983). 0M male foetuses are exposed to the highest titers of estradiol with the result that they behave as if they had been exposed to much lower titers of testosterone during foetal life than 2M males. Specifically, 0M males are less likely to exhibit aggression and more likely to commit infanticide when treated with testosterone in adulthood than are 2M males. In contrast to the antagonistic effect of estrogen on the action of testosterone in the neural areas mediating infanticide and intermale aggression, estrogens appear to facilitate the effects of testosterone in the neural areas mediating male sexual behavior (mounting, intromitting, and ejaculating). Thus, in adulthood, 0M males are more sexually active than are 2M males in both mice and rats (vom Saal *et al.*, 1983). In summary, during foetal life, the interaction of circulating estrogens and androgens in different neural areas is quite complex. Such differences may be determined by the type of steroid receptors present in particular neural areas during foetal life.

In humans, female foetuses also have higher titers of estrogens than do male foetuses, and this difference is due to sex differences in the secretion of adrenal androgens, which are converted to estrogens in the placenta (Belisle and Tulchinsky, 1980; Winter, Fujieda, Fatman, Reyes, and Thliveris, 1980). The source of estradiol in both the foetal and maternal circulation during pregnancy in mice is unknown. But, the available evidence suggests that it is also of foetal/placental origin, since female mouse foetuses have over twice the circulating titers of estradiol as do their mothers 2 days prior to parturition (vom Saal and Bronson, 1980a). In mice, both sex differences in behavior and variation among males and among females due to intrauterine position may thus result from an interaction of circulating estrogens (possibly derived from adrenal secretions) and androgens.

Intrauterine Position in Relation to the Effect of Mating on Infanticide

Intrauterine position influences the behavior of male mice toward young, and it is obviously of interest to determine whether there is

an interaction between IUP and the inhibition of infanticide that occurs between 12 and 50 days after mating in male mice. For example, after mating, not all males behave parentally, and these males may be 0M males, which have a tendency toward committing infanticide. Similarly, after achieving dominance, not all males commit infanticide, and these males may be 2M males. Such a finding would suggest that exposure to different concentrations of hormones during foetal life due to IUP results in an animal being biased toward a particular behavior pattern that cannot be easily altered by postnatal experiences. For example, it is interesting that in an experiment in which colony males were pretested for their behavior toward young and then retested at either 1 or 20 days after mating (see Table II), all of the males that did not commit infanticide 1 day after mating (17% parented or ignored the young) had not committed infanticide on the pretest. Similarly, all of the males that did not behave parentally 20 days after mating (23% committed infanticide) had also committed infanticide on the pretest. Since 0M and 2M males each represent about 20–25% of a population of males (50% of a population of males are 1M males; vom Saal, 1981), a group of 30 males chosen at random with respect to IUP would contain about 7 0M males (23%) and 7 2M males, figures that are very close to those obtained previously. These observations provide indirect evidence that IUP may render male mice differentially sensitive to the short-term facilitating and inhibiting effects of mating on infanticide and parental behavior. In contrast, this author has previously tended to view IUP effects on behavior as biases that could be overridden by postnatal experiences.

ECOLOGICAL CONSIDERATIONS

It is an open question as to what significance findings from studies in the laboratory have for the understanding of the reproductive ecology of wild animals. But, it is not unreasonable to speculate concerning the possible significance of some of the findings described in this chapter in terms of individual reproductive success and population dynamics in wild mice. For example, from an evolutionary perspective, it is quite interesting that foetal hormones act in opposite ways on infanticide and intermale aggressive behavior. Intuitively, these behaviors would seem likely to be positively rather than negatively correlated.

Mice have a period of postpartum estrus and thus have the capacity to produce litters every 3 weeks. If many young survive, a mouse plague can occur. When population density begins increasing and competition for space within the natal territory becomes intense, the aggressive animals within a deme are believed to drive the less aggressive animals

away (Lidicker, 1975). When the one dominant male within a deme dies, the subordinate mice will fight until one male becomes dominant. Previously discussed was the likelihood that subordinate males within a deme will be brothers, half-brothers, or offspring of the dominant male, since immigration of males into a deme has been reported to be uncommon (Reimer and Petras, 1967; Lidicker, 1976). The subordinate males will also be related to many females within a deme. It is proposed that there may be a strong selective advantage for males that have the highest sensitivity to the activational effects of testosterone on intermale aggression (i.e., the 2M males) to also have the lowest tendency toward infanticide, since these same males may very well be the most likely to become the dominant male in their natal territory and thus come in contact with genetically related young prior to, themselves, mating. Since there is an increase in the tendency of sexually naïve male mice to commit infanticide after fighting and achieving dominance (see Table IV), a corollary of this hypothesis is that after fighting and achieving dominance, 2M males will still be less likely to commit infanticide than will 0M males (see foregoing).

The finding that 2M males are more aggressive than 0M males after treatment with testosterone suggests that in an environment in which 0M and 2M males were in direct competition, the 0M males should have a greater likelihood of being driven out of the natal environment than the 2M males. The dispersing 0M males that survive (*Mus* are highly successful colonizers) and come in contact with a female with newborn young may be more likely than would 2M males to kill the female's litter and then mate with her. It would be unlikely that the dispersing 0M males would be related genetically to females or young encountered outside of the male's natal territory, and committing infanticide in this situation should result in an increase in the male's fitness. It must be emphasized that the preceding discussion is simply an attempt to provide a plausible hypothesis concerning the observed inverse relationship between intermale aggressiveness and infanticide in 0M and 2M male mice (Table VII).

SUMMARY

The studies described here provide support for two predictions of the sexual selection hypothesis, namely, that there has to be a method for the inhibition of infanticide in situations where a male may possibly kill his own offspring, and that males must potentially obtain an increase in reproductive success as a result of committing infanticide. These experiments have not directly addressed the third prediction that some component of the variance in the tendency of male mice to commit infanticide or behave parentally should be mediated geneti-

cally. But, a significant component of the variance in the tendency of male mice to commit infanticide can be accounted for based on intrauterine position, adult dominance status, and prior mating experience. An important aspect of the finding that behaviors such as aggression and infanticide are modulated by prenatal hormone levels in mice is that regardless of the amount of genetic variation within a population, animals in the population will be guaranteed to vary in terms of these behaviors due to the intrauterine position phenomenon.

The overwhelming complexity of the regulation of infanticide by prenatal hormones and then by adult experience is such that the process by which selection could have operated to produce such a system is difficult to imagine. However, it is quite likely that a system that is this complex is mediated by a large array of genes. In the process of attempting to develop infanticidal and noninfanticidal mouse strains through selective breeding, shifts in the frequencies of genes involved in regulating many other traits, in addition to infanticide, will thus likely occur. While the issue of the heritability of infanticide needs to be examined using the techniques of behavior genetics, this will undoubtedly be a difficult task (see Svare *et al.*, Chapter 20, this volume).

In summary, the hypothesis that infanticide is a pathological behavior only observed during times of social stress appears to be untenable, although it is recognized that social stress can result in an increase in infanticide in mouse populations (Christian, 1971) and may be a factor in influencing infanticide in other species as well (Hrdy, 1979). In species that occupy different ecological niches and that have different social structures from mice, other variables will certainly be found to influence infanticide. However, as has proved to be the case with mice, information concerning the reproductive physiology and socioecology of a particular species is essential for understanding which variables play a role in regulating infanticide. The relative lack of such information for most mammals in comparison to what is known about the genetics, physiology, and ecology of house mice will make examining the proximate causes of infanticide in other species as well as speculating about ultimate causation far more difficult than has been the case with mice.