

Susan W. Margulis

Inbreeding-based bias in parental responsiveness to litters of oldfield mice

Received: 20 September 1996 / Accepted after revision: 28 April 1997

Abstract This study investigated variation in parental behavior in oldfield mice (*Peromyscus polionotus*). Specifically, I examined the possibility that parental behavior and probability of litter loss may differ depending on whether parents are related to one another and, therefore, whether offspring are inbred. When parents are related, they share more genes with their offspring and so may be predicted to invest more in inbred offspring if these offspring do not inherently have a greater risk of juvenile mortality. Survival of inbred pups did not differ from survival of outbred pups, and females tended to exhibit a preference, in the form of enhanced parental care, for inbred litters. Males did not display any consistent preferences. Inbred litters were left unattended more often during the immediate post-partum period than were outbred litters, but this inconsistent finding appeared to have a smaller effect than the overall improved maternal care provided to inbred litters. Females thus appeared to modulate their behavior to a greater degree than did males depending on characteristics of the litter. Patterns of litter loss indicated that complete death of litters was independent of litter inbreeding, whereas loss of selected pups in a litter occurred significantly more often when litters were inbred. Complete and partial loss of litters may be functionally different behaviors that are triggered by different cues.

Key words Parental behavior · Inbreeding · Oldfield mouse · *Peromyscus polionotus*

Introduction

In species with parental care, and especially those with biparental care, failure of an offspring to survive may reflect a deficit in the offspring itself, or may reflect inadequate care on the part of the parents. Successful reproduction depends both on the individual traits of several parties – father, mother, and offspring – and on their interactions. That reproductive success is a function of both parental phenotypes and offspring phenotypes has recently been incorporated into population genetics models (Kirkpatrick and Lande 1989), an area in which research has typically focused on the fitness consequences of genotypes of only a single generation.

When relatives mate and produce inbred offspring, decreased viability of progeny is often the result. Darwin (1872) stated in *The Origin of Species* (sixth edition) that mating between close relatives “...almost always leads to decreased size, weakness, or sterility,” and that inbreeding is generally avoided in nature. Most commonly, inbreeding depression is measured as the decrease in the viability of inbred offspring (Ralls et al. 1979; Ballou and Ralls 1982; Ralls and Ballou 1982a,b; Brewer et al. 1990). In this context, inbreeding depression affects fitness because inbred offspring, produced when relatives mate, have a lower probability of survival than do outbred offspring.

Thus, when an inbred offspring fails to survive, its death is commonly attributed to an inherent deficit in the inbred offspring itself (e.g., through exposure of deleterious recessive traits that are commonly masked in outbred offspring). Another possibility, however, is that death of an inbred offspring may reflect altered parental responsiveness towards inbred offspring.

We might predict that parents should limit their investment in inbred offspring because we might expect these offspring to inherently have a lower probability of survival than outbred offspring. On the other hand, we might instead expect parents to direct more care towards inbred offspring, because parents share a greater

S.W. Margulis (✉)
Committee on Evolutionary Biology, University of Chicago,
940 E. 57th Street, Chicago, IL 60637, USA
Fax: (773) 667-3779; e-mail: swm4@midway.uchicago.edu

S.W. Margulis
Department of Conservation Biology, Chicago Zoological Society,
Brookfield, IL 60513, USA

proportion of genes with their offspring when mates are related (i.e., when the offspring are inbred), than when mates are not related to one another. In the case of a mating between full siblings, for example, parents share three-quarters of their genes with their offspring. In contrast, mates that are not related share half their genes with their progeny. Thus, inbred offspring, if they are not inherently at higher risk of juvenile mortality, represent a greater fitness gain than do outbred offspring. This has been suggested as a possible strategy in mammalian populations in which inbreeding is unavoidable or in which the costs of migration exceed the costs of inbreeding (Smith 1979; Chesser and Ryman 1986). Furthermore, if outbred offspring are presumed to be inherently healthier than inbred infants, outbred infants may not benefit from more care, while the added investment in weak but retrievable inbred infants may make the difference between offspring loss and survival.

In the present study I investigate two aspects of parental behavior in captive oldfield mice, *Peromyscus polionotus*. Previously, I demonstrated that litter survival in this captive population is influenced by levels of parental care, particularly maternal care, and that survivorship of inbred and outbred litters does not differ (Margulis 1996, 1997a,b). In the present study, I first address the question of whether parents vary their behavior depending on whether they are related to one another (i.e., whether their litter is inbred).

Second, I examine a subset of parental behavior that deserves special attention because of its extreme and obvious fitness consequences. Destruction of conspecific young has been reported anecdotally in vertebrate populations for a considerable time, but only recently has this behavior, under some circumstances, come to be viewed as an adaptive response (see for example Hrdy 1979; Packer and Pusey 1983a,b; Mock 1984). Parental infanticide (destruction of one's own young) is more unusual, but is not uncommon among small rodents, particularly in captivity (Brooks 1984). In this context, parental infanticide is often thought to be a response to stress (Layne 1968), an effort to "cut one's losses" when the probability of litter survival is low (Clutton-Brock 1991), or to reduce the size of large litters to some optimal, or at least manageable, level (Day and Galef 1977; Gandelman and Simon 1978). While it was not possible to distinguish between pup loss due to natural causes (weak or stillborn pups, lactational failure) and active destruction and cannibalism of pups, I nonetheless make an important distinction between loss of a litter in its entirety, and loss of selected pups within a litter.

If loss of litters in their entirety is a parental response to stress, we would not expect to see any difference in the frequency of complete litter loss for inbred and outbred litters. Different predictions arise when some, but not all pups in a litter die prior to weaning. When parents are related, their offspring will be inbred, and might be more likely to express deleterious recessive traits than would outbred offspring. However, not all inbred offspring will

necessarily express detrimental traits. Thus, if loss of pups, possibly due to failure of parents to invest in them or actual destruction of pups, is an adaptive response on the part of the parent, we would expect death of some but not all pups in a litter to occur more often when litters are inbred. If we find no difference in the occurrence of total litter loss for inbred and outbred litters, but more frequent partial loss of inbred litters, this would support the contention that these two types of litter loss are quite different behaviors that may be responses to different cues.

Methods

Subjects

Two subspecies of the oldfield mouse, *Peromyscus polionotus*, were subjects of this study. This nocturnal species is believed to be monogamous, and both parents contribute to the care of the young (Smith 1966; Foltz 1979, 1981; D. Gubernick and C. Dold, unpublished work). *P. polionotus subgriseus* were captured from the Ocala National Forest in north-central Florida in 1990. *P. polionotus rhoadsi* were captured near the town of Lake Placid, Florida in 1990 and 1991 (Lacy et al. 1996). Captive stocks of both populations have been maintained at Brookfield Zoo, and have been the subject of intensive genetic study by Lacy and co-workers (Brewer et al. 1990; Lacy 1992; Lacy et al. 1996). As part of ongoing genetic analyses, detailed pedigrees of all animals have been maintained by Dr. Robert Lacy. I could therefore determine the inbreeding coefficients of all animals in the present study, as well as of their prospective offspring. Subjects were sixth through eighth generation captive-born. All wild-caught founders were assumed to be unrelated to one another.

Experimental design

The study group consisted of 165 *Peromyscus polionotus subgriseus* pairs and 167 *P. p. rhoadsi* pairs, divided into eight experimental groups based on maternal inbreeding, paternal inbreeding, and the inbreeding coefficient of future litters, if produced (Table 1). For the purposes of experimental design, I considered animals with inbreeding coefficients greater than 0.1 to be inbred. This breeding

Table 1 Experimental design and sample sizes. Numbers in parentheses are the number of pairs in each category that produced litters [*i* inbred ($f > 0.1$) (range 0.1010–0.5859), *o* outbred ($f \leq 0.1$) (range 0–0.0980)]

Experimental groups			<i>P.p. subgriseus</i>	<i>P.p. rhoadsi</i>
Maternal inbreeding	Paternal inbreeding	Litter inbreeding	<i>n</i>	<i>n</i>
<i>i</i>	<i>i</i>	<i>i</i>	21 (8)	22 (15)
<i>i</i>	<i>i</i>	<i>o</i>	20 (13)	21 (10)
<i>o</i>	<i>o</i>	<i>i</i>	21 (11)	20 (18)
<i>o</i>	<i>o</i>	<i>o</i>	21 (11)	22 (15)
<i>o</i>	<i>i</i>	<i>i</i>	20 (11)	21 (16)
<i>o</i>	<i>i</i>	<i>o</i>	20 (8)	20 (15)
<i>i</i>	<i>o</i>	<i>i</i>	21 (14)	21 (15)
<i>i</i>	<i>o</i>	<i>o</i>	21 (14)	20 (13)
			165 (90)	167 (117)

design allowed me to separate the effects of inbreeding on the parent from inbreeding on the litter. In no case were littermates paired.

Pairs were housed in standard polycarbonate mouse cages, and received mouse chow and water ad lib. They were maintained on a 12:12 light cycle, with the light phase beginning at 0600 hours CST. Red lights (40 W) provided dim illumination during the dark phase. All subjects were at least 55 days of age at the time of pairing. Pups were weaned at 20 days of age, ear-punched for identification, and weighed. Gestation is believed to be 23–24 days (Smith 1966). *P. polionotus* usually undergoes a post-partum estrus, thus females typically produce another litter within a week of the removal of a weaned litter (inter-litter interval = 28.8 ± 0.34 days [mean \pm SE], $n = 618$ inter-litter intervals in the present series of studies). I separated pairs at the weaning of the third litter, or by 120 days if no litter was present at that time or if the pair failed to breed entirely. A pair could thus produce a maximum of four litters during the course of the study, with the male present for the rearing of up to three litters.

The main analysis included data on all litters (excluding first and last litters, which were analyzed separately) from the 207 pairs that reproduced and for which both parents were present during the period of parental care ($n = 352$ litters). Because the two subspecies did not exhibit significant differences in parental behavior, the data were pooled. Where possible, when data were available for more than one litter per pair, I averaged the data to yield a single value for each pair. As is common in mammals, parental behavior and litter viability differed between first versus subsequent litters (e.g., Wang and Nowak 1994). I therefore present results for first litters ($n = 189$ litters) separately. In addition, because the study design resulted in females rearing their final litter without their mate present ($n = 203$ litters), these litters too, were analyzed separately, and I refer to them for comparisons. The main report as a whole therefore, derives from the 352 litters from 207 pairs, unless otherwise indicated.

Data collection

To facilitate identification of subjects during behavioral observation, I dyed one member of each pair with black hair dye several days before pairing (Clairol Nice 'n Easy hair dye). In half the pairs I dyed the male, and in half the female. This dye has been used on this and related species with no apparent effect on behavior (D. Gubernick, personal communication; S. Margulis, personal observations). I used a Psion palm-top computer to record the data, and all behavioral observations were conducted without observer knowledge of inbreeding level of subjects, or the relationship of pair members to one another.

Peromyscus litters are usually born during daylight hours, i.e. during the inactive period (Layne 1968). Beginning 20 days after pairing (4–5 days before any litters would be expected), I checked cages for the presence of litters between 0900 hours and 1100 hours each morning, and again in the late afternoon. Upon discovery of a litter, I noted initial litter size and litter number, and conducted a 20-minute focal observation (Altmann 1974) of the parents and neonates. Behaviors recorded included contact with pup, nuzzling, carrying pup, and nest-building (see ethogram in Margulis 1996). An unpublished pilot study indicated that the amount of time that parents spent in contact with pups, and number of nuzzles pups received during the immediate post-partum period, were positively associated with pup survival for primiparous pairs (M. Glymour, unpublished work). After 10 min, I added cotton nesting material to the food hopper, and collected an additional ten minutes of data. All pairs that were given cotton built nests by the end of the light period, although not all pairs did so during the 10-min observation period. During the second 10 min of observation, I recorded total time spent nest-building, as well as the latency to begin nest-building. Only litters containing at least one live pup at the time of observation (i.e., the litter had not been completely lost), were included in the behavioral analyses.

Based on casual observations suggesting that nest quality was a good indicator of litter survival, I evaluated nest quality beginning

on the day following the birth of the litter (day 1), and subsequently on days 2, 5, 10 and 20. Specifically, litters were more likely to survive when nests were high (i.e., were completely covered on top and extended to the lid of the cage), and when less than half the cage floor was covered by nesting material. In an effort to quantify this phenomenon, I evaluated nest quality on a scale of 1–4, with 1 being a poor nest (low, covering more than half of the cage floor), and 4 a nest of high quality (maximal height, covering less than half of the cage floor). Nest height and area were measured to the nearest 0.5 cm. All nest measurements were approximate, and were made from outside the cage, in order to minimize disturbance to the litter. I assessed 751 nests.

In order to obtain a broad picture of activity throughout the whole 20-day period of parental care, I made twice-weekly scans on all 207 pairs that produced at least one litter. Because *Peromyscus* are nocturnal, I collected these behavioral data when the animals were active, during the lights-off period. In addition to the red lights, I used a miner's headlamp fitted with a red filter during these nocturnal observations. I carried out scan samples (Altmann 1974) for 1 h, with scans at 15-min intervals. On each scan, I collected three types of data: first, whether the members of the pair were in contact, second, whether one or both pair members were in the nest with the pups (when pups were present), and finally, the activity of the two adults (see Margulis 1996 for complete ethogram). If parents were in the nest, it was not possible to observe their behavior. I assumed that (1) a parent in the nest was in contact with the litter; and (2) both parents simultaneously in the nest were in contact with the litter and with each other. Occasional nest-checks, during which I parted the cotton sufficiently to see into the nest, confirmed that this was so. In order to pool data that were collected on females that may or may not have been pregnant (with their post-partum litter) during observations, and may have been caring for litters of varying age (0–20 days), I first ascertained that neither litter age (0–20 days old) nor stage of pregnancy significantly affected female behavior. Raw data were converted into per cent time each subject spent engaging in each behavior (active, inactive, nest-building, carrying pup; see Margulis 1996 for complete ethogram), per cent time in contact with mate, and per cent time in nest with pups, when pups were present. Per cent time spent in nest, in contact with pup, and inactive were highly correlated with one another (r ranged from 0.91 to 1.0). Therefore, I analyzed only per cent time in nest.

Data analysis

I used nonparametric statistics (Kruskal-Wallis test, SAS NPARIWAY procedure, SAS 1988b) to evaluate the effect of parental relatedness (i.e., litter inbreeding) on parental behavior (proportion of time spent in contact with pups, nest-building, carrying pups, and frequency of pup-nuzzling). When appropriate, I calculated means for each pair ($n = 207$ pairs that produced litters). I used survival analysis to examine the effect of litter inbreeding on latency to begin nest-building (Kalbfleisch and Prentice 1980; SAS LIFETEST procedure, SAS 1988a). Survival analysis is applicable to failure-time data, such as is typically found in biomedical research (Kalbfleisch and Prentice 1980). The endpoint of failure-time data is time until an event occurs. Such studies will include subjects who have not "failed" (i.e., have not reached the endpoint) at the end of the study period, or who have been removed from or left the study before its end. Unlike most statistical techniques, survival analysis allows these incomplete values to be included in the analyses as "censored" data. In the present study, the endpoint in question is the onset of nest-building. Censored data arose from pairs that failed to begin nest-building by the end of the 10-minute observation period. In order to analyze the effect of inbreeding on nest quality using logistic regression, I rescored the subjective nest quality rating dichotomously as 0, low-quality, for nests rated as quality = 1 or 2, and 1, high quality, if the quality rating was 3 or 4. I then performed logistic regression (Hosmer and Lemeshow 1989; SAS LOGISTIC procedure, SAS 1990) to assess the effect of litter inbreeding on nest quality. Logistic regression fits data to models of the form: $P = e^{a+bx} /$

$(1 + e^{a+bx})$, in which P is the probability of the response variable (i.e., viability or nest quality), x is the predictor variable(s) (litter inbreeding), and a and b are fitted regression coefficients. The significance of each predictor variable is tested using a Wald χ^2 statistic, comparing the model with and without the predictor variable (SAS LOGISTIC procedure, SAS 1990).

I included all litters in the analyses of litter loss. Only rarely were parents observed in the act of killing pups; more commonly, partially eaten pups were found in the cage. Therefore I could not distinguish between pups that were killed by parents and pups that died and were then eaten. Of a total of 870 litters, 357 litters were fully or partially lost. Of these, 237 litters from 135 pairs were fully lost, and 120 litters from 83 pairs were partially lost. In order to control for the tendency of some pairs to repeatedly lose litters, I used the pair, and not the litter, as the unit of analysis. A pair could, in theory, be represented in both the complete and partial loss categories, but only a single time in each. A pair that completely lost two litters and partially lost one litter for example, would be represented a single time in each of the two loss categories. I used chi-square tests to evaluate differences in the frequency of partial and complete litter loss when litters were inbred (i.e., when parents were related), and when litters were outbred (parents were not related). This was done for all litters, and repeated excluding first litters and last litters. I evaluated litter loss patterns for first and last litters separately for comparative purposes.

Results

Parental behavior differed significantly toward inbred and outbred litters during the immediate post-partum period, but not during the nocturnal scans conducted throughout the twenty days of parental care. During the immediate post-partum period, dams nuzzled inbred pups significantly more often than they nuzzled outbred pups (Kruskal-Wallis test, $\chi^2 = 5.75$, $df = 1$, $P < 0.02$), and they spent significantly more time carrying inbred pups ($\chi^2 = 13.06$, $df = 1$, $P < 0.0003$). Sires spent significantly more time in contact with outbred pups than inbred pups ($\chi^2 = 4.25$, $df = 1$, $P < 0.04$). Inbred litters were left unattended significantly more often than were outbred litters ($\chi^2 = 4.92$, $df = 1$, $P < 0.03$). Sires rarely spent time in contact with their litters when their mate was not also in contact with the litter (sires were in contact with their litters without their mate for only 2.9% of the 10-minute post-partum observation; dams were in contact with their litters without their mate 24% of the time) (Fig. 1).

Dams had significantly shorter latencies to begin nest-building when litters were inbred (survival analysis, $\chi^2 = 4.88$, $df = 1$, $P < 0.03$). Sire latency did not differ for inbred and outbred litters ($\chi^2 = 1.98$, $P < 0.16$) (Fig. 2). Previously, I found that maternal inbreeding influences behavior (Margulis 1996, 1997b). When inbreeding coefficient of the parent is also taken into account (i.e., whether parents were themselves inbred), inbred dams with inbred litters had significantly shorter latencies to begin nest-building (inbred dams: $\chi^2 = 7.22$, $df = 3$, $P < 0.007$, $n = 73$ outbred litters, 90 inbred litters; outbred dams: $\chi^2 = 0.29$, $df = 3$, $P < 0.6$, $n = 89$ outbred litters, 100 inbred litters). In contrast, outbred sires had significantly shorter latencies when their litters were inbred (outbred sires: $\chi^2 = 4.56$, $df = 3$, $P < 0.03$,

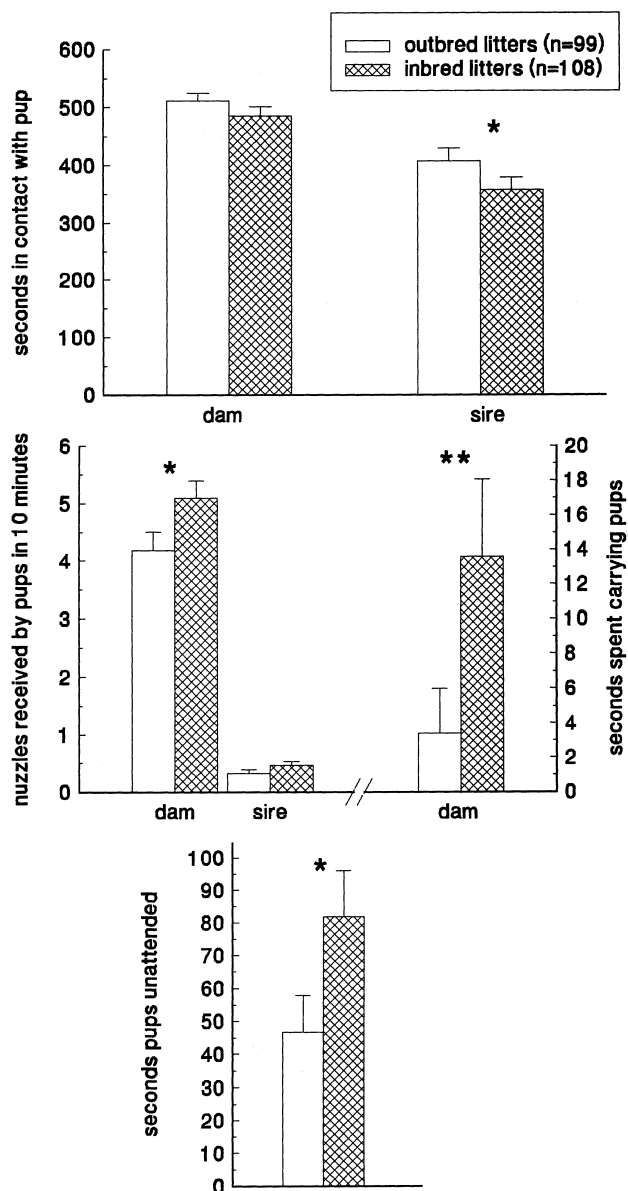


Fig. 1 Parental behavior towards inbred and outbred litters during the post-partum period. *Upper panel*: seconds (out of 600) dam and sire spent in contact with pup. *Middle panel*: number of times dams and sires nuzzled pups, and number of seconds (out of 600) dams spent carrying pups (sires were observed to carry pups too rarely to be analyzed). *Lower panel*: Seconds (out of 600) that both parents spent together in contact with the litter, and seconds that litters were left unattended by both parents. * $P < 0.05$; ** $P < 0.003$

$n = 79$ outbred litters, 100 inbred litters; outbred sires: $\chi^2 = 0.26$, $df = 3$, $P < 0.6$, $n = 83$ outbred litters, 90 inbred litters).

I found no differences for dams in any of the behaviors described above during the post-partum period for first litters based on whether mates were related (i.e., litters were inbred). Sires however, spent significantly more time nest-building following the birth of first litters when litters were inbred ($\chi^2 = 5.65$, $df = 1$, $P < 0.02$). Dam latencies to begin nest-building did not differ for

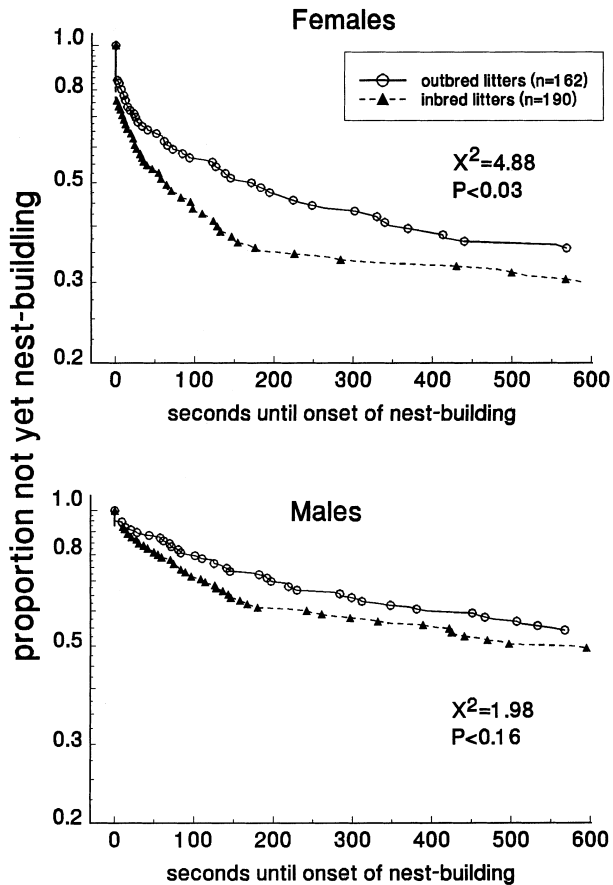


Fig. 2 Latency to begin nest-building by dams and sires. Those subjects that failed to begin nest-building at the end of the 10-minute observation period were included as “censored” observations (33% of dams, 52% of sires)

first litters ($X^2 = 0.42$, $df = 1$, $P < 0.35$). However sires had significantly shorter latencies for first litters when their litters were inbred ($X^2 = 4.11$, $df = 1$, $P < 0.04$).

Nest quality on the day following parturition was significantly worse when litters were inbred (logistic regression, Wald $X^2 = 6.36$, $df = 1$, $P < 0.01$). By day two, and on all subsequent nest assessments, nest quality did not differ for inbred and outbred litters.

A total of 135 pairs completely lost at least one of their litters ($n = 237$ litters). Pairs that were related to one another (i.e., litters were inbred) were no more likely to lose their entire litters than were unrelated pairs ($X^2 = 0.6$, $df = 1$, $P > 0.1$). This pattern was consistent for first litters, litters in which only the female was present, and all other litters (Table 2).

Partial litter loss occurred significantly more often when pairs were related (litters were inbred) than when pairs were not related ($X^2 = 4.35$, $df = 1$, $P < 0.05$). This difference remained significant when first litters, and litters in which the sire was not present, were excluded ($X^2 = 6.40$, $df = 1$, $P < 0.025$), and approached significance for first litters alone ($X^2 = 2.79$, $df = 1$, $P < 0.1$). The difference was not significant for litters in

Table 2 Results of analysis of total and partial cannibalism of litters. Data are presented for all litters, as well as broken down for first litters, litters in which the male was not present at the time of parturition, and litters after the first litter in which both parents were present. Because some pairs had a tendency to repeatedly cannibalize litters, each pair that cannibalized a litter, rather than each litter that was cannibalized, was used as the unit of analysis (note: if each litter is used as an independent data point, the results for partially cannibalized litters are strengthened, whereas no change is seen for fully cannibalized litters)

	Inbred litters	Outbred litters	X^2	P
Fully cannibalized litters:				
All litters (135 pairs)	72	63	0.60	>0.1
First litters only (94 pairs)	45	49	0.17	>0.5
Litters without male present (30 pairs)	19	11	2.13	>0.1
Remaining litters (82 pairs)	43	39	0.20	>0.5
Partially cannibalized litters:				
All litters (83 pairs)	51	32	4.35	<0.05
First litters only (29 pairs)	19	10	2.79	<0.1
Litters without males present (35 pairs)	22	13	2.31	>0.1
Remaining litters (45 pairs)	31	14	6.40	<0.025

which only the dam was present ($X^2 = 2.31$, $df = 1$, $0.1 < P < 0.5$), although the trend was in the same direction (Table 2).

The initial litter size of litters that experienced loss of some, but not all pups, did not differ from the initial litter size of litters that did not experience any pup loss (mean \pm SE = 4.53 ± 0.14 pups for litters with no loss, 4.36 ± 0.06 pups for litters with partial loss). Significantly fewer pups survived to weaning in litters in which some pups were lost (3.56 ± 0.09) than in litters that experienced no pup loss (4.36 ± 0.06). I found no differences in the initial size of outbred and inbred litters (4.21 ± 0.06 for outbred litters; 4.21 ± 0.07 for inbred litters).

Discussion

Female oldfield mice tended to exhibit improved parental behavior during the immediate post-partum period when the females were related to their mates and their litters were therefore inbred. In contrast, male behavior was less variable, with males tending to show either no difference, or worse parental care towards inbred litters (Table 3). Previously, I reported that dams performed more parental behavior during the immediate post-partum period than did sires, and female behavior significantly affected pup survival to a greater degree than did male behavior (Margulis 1996, 1997b). Thus deficits in male parental behavior do not put litters at

Table 3 Summary of results, indicating direction of response. A plus (+) indicates that the behavior was directed more often towards litters in that inbreeding category, a minus (–) less often. An equal sign (=) indicates no difference. Because a shorter latency to begin nest-building is the preferred behavior, + and – are not used in this category

Behavior	Inbred litters	Outbred litters
Mother nuzzles pup	+	–
Mother carries pup	+	–
Mother in contact with litter	=	=
Father in contact with litter	–	+
Litter unattended	+	–
Mother's latency to begin nest-building	shorter	longer
Father's latency to begin nest-building	=	=
Nest quality on day 1	–	+
Nest quality after day 1	=	=
Complete litter loss	=	=
Partial litter loss	+	–

increased risk. Additionally, and surprisingly, I found that inbred pups were as likely to survive as outbred pups, and in the *P. p. rhoadsi* subspecies, were significantly more likely to survive (Margulis 1996, 1997a). The results of the present study, indicating that females tend to show improved parental care when their offspring are inbred, are consistent with these earlier findings. They further suggest that females modulated their behavior to a greater degree than did males, based on whether their litters were inbred.

One inconsistent finding, however, was that inbred litters were left unattended significantly more often during the immediate post-partum period than were outbred litters. Despite this finding, these litters nevertheless did not exhibit any decreased survival probability (Margulis 1997a). Females may have left litters unattended for a variety of reasons during this brief 10-minute period, including increased activity prior to beginning nest-building, occasional chasing of the mate, or retrieving scattered pups. The improved maternal care directed towards inbred litters has a greater impact on litter survival, and appears to be a more significant finding than the increased tendency for inbred litters to be left unattended.

When parental inbreeding level is overlaid on the inbreeding status of the litter, inbred females exhibited shorter latencies to begin nest-building when their litters were inbred, whereas inbred males had longer latencies when their litters were inbred. These results too, are in agreement with previous findings in which inbred females showed a tendency to exhibit slightly improved parental care behaviors, whereas inbred males exhibited significantly poorer nest-building than did outbred males (Margulis 1996).

Females appear to respond to differences in pup quality or survival probability to a greater degree than do males. This may be so for several reasons. First, females have greater certainty of parentage than do males, even in monogamous species. Some low level of extra-

pair copulation may occur in natural populations (Foltz 1981). Thus females have more to gain by investing more in parental care than do males. Second, females have a greater metabolic investment in litters than do males, and are expected to invest in pups in such a way as to maximize their probability of survival. Under some circumstances, this may mean investing in some, but not all pups in a litter. The results of the partial litter loss analyses are in agreement with this assumption. *Mus musculus* females for example, selectively cannibalize small pups (Gandelman and Simon 1978), which presumably have a lower probability of survival. Finally, both males and females will share more genes with their offspring when they are related to their mates (although males will, in the wild, still face the question of paternity certainty), and might therefore be expected to invest more in their litters than unrelated mates.

The litter loss rates observed in the present study (27% of litters were completely lost) are consistent with overall loss rates observed for *Peromyscus polionotus* (18–26%) during long-term demographic monitoring in the Brookfield mouse colony (R. Lacy, personal communication). Comparable data from other captive colonies are limited. Rood (1966) reported litter loss rates of 37% for *P. polionotus* in a captive colony at Michigan State University. Whether litter loss rates are higher in the Brookfield colony than in other *Peromyscus* colonies cannot be determined from the present data. However, even within the Brookfield colony, loss rates varied considerably among species, and among subspecies. Further, because reproductive failure is a key variable under investigation in the Brookfield colony, pairs that fail to breed or consistently lose litters are not removed from the breeding program, as they might be in colonies whose primary function is to produce animals.

Complete loss of litters was independent of litter inbreeding, whereas partial litter loss occurred significantly more often among inbred litters than outbred litters. This leads to the conclusion that these two types of pup loss may be triggered by different cues and may in fact have quite different mechanisms. Complete litter loss – i.e., complete loss of one's reproductive effort – may in fact be an aberrant behavior in *P. polionotus*, or a behavior induced by social stress or other extrinsic factors characteristic of captivity, and is likely independent of pup quality or viability. In contrast, partial litter loss may be at least in part a response to differences in pup quality or behavior. These findings, together with the observed differences in parental behavior towards inbred and outbred litters, suggest that parents may be able to detect differences among individual pups in a litter and may be ceasing investment in or perhaps actively destroying those pups that exhibit deficits, causing these pups to be poor competitors or to fail to perform behaviors that elicit appropriate parental responses.

While I cannot confirm with the data presented here that inbred pups are more likely to exhibit abnormalities or deficits of some kind, the long-term genetic studies on these captive populations suggest that inbred individuals

are more likely than outbred animals to express deleterious traits, such as lower weaning mass (Brewer et al. 1990; Lacy et al. 1996) and greater fluctuating asymmetry in skeletal measurements (R.C. Lacy, G. Alaks and A. Walsh, unpublished work). In addition, inbred individuals may experience deficits that are not measurable until adulthood, such as reduced fertility (Margulis and Altmann 1997).

The ability to extrapolate from the present findings to other mammalian species must await the collection of additional data on both captive and wild populations. However, the potential applicability of this research to captive breeding programs, or interventive management of wild populations in which inbreeding is often unavoidable, may be substantial. Death of inbred offspring may be attributed not only to the expression of deleterious recessive alleles in inbred individuals, but also to variations in parental responsiveness towards offspring. First, the tendency for studies to focus on neonatal and juvenile mortality as indicators of the occurrence of inbreeding depression ignores the interaction between offspring behavior and parental response. Second, parents may modify their behavior based on cues that they receive from their offspring. Lacy et al. (1996) found that inbred *P. polionotus* litters exhibited greater within-litter variance in pup weight, suggesting greater within-litter heterogeneity in pup quality when litters are inbred, perhaps leading to greater within-litter variation in parental responsiveness. Finally, parents may be selectively rearing those offspring with the best prognosis for long-term survival. Unlike complete loss of a litter, the death of some but not all pups in a litter – presumably those with a low probability of survival – may be an adaptive response on the part of the parent (e.g., Gandelman and Simon 1978). Thus, artificial attempts to hand-rear offspring that have been rejected by parents may in fact reduce the overall fitness of the captive population by perpetuating deleterious genes and promoting poor parental behavior. While the general applicability of the present findings must await further investigation, it is nonetheless important to recognize and continue to examine the role that the parent-offspring interaction may play in survival of a litter.

Acknowledgements This research was motivated by a pilot study conducted by University of Chicago undergraduate Maria Glymour. Many thanks to my advisor, Jeanne Altmann, for her insights and input into this research. I thank research technicians Glen Alaks and Allison Walsh for providing excellent care to the animals. Michael Wilson and Dominique Shimizu assisted in all aspects of data collection. Peter Margulis provided exceptional programming assistance. Ted Karrison offered assistance and guidance in the use and interpretation of logistic regression analyses. Lee Drickamer, Jack King, Bob Lacy, James Layne, Martha McClintock, Carole Ober, Chung-I Wu, and two anonymous reviewers provided useful comments on the manuscript. This research was supported by grants from the Chicago Zoological Society, Sigma Xi, the Animal Behavior Society, the University of Chicago Hinds Fund, and NSF Doctoral Dissertation Improvement Grant IBN-9321187. The author was supported by a Luce Foundation Fellowship, a Genetics and Regulation Training Grant to the University of Chicago, and a University of Chicago Harper Fellowship.

References

- Altmann J (1974) Observational study of behavior: sampling methods. *Behaviour* 49:227–267
- Ballou J, Ralls K (1982) Inbreeding and juvenile mortality in small populations of ungulates: a detailed analysis. *Biol Conserv* 24:239–272
- Brewer BA, Lacy RC, Foster ML, Alaks G (1990) Inbreeding depression in insular and central populations of *Peromyscus* mice. *J Hered* 8:257–266
- Brooks RJ (1984) Causes and consequences of infanticide in populations of rodents. In: Hausfater G, Hrdy SB (eds) *Infanticide: comparative and evolutionary perspectives*. Aldine, New York, pp 331–348
- Chesser RK, Ryman N (1986) Inbreeding as a strategy in subdivided populations. *Evolution* 40:616–624
- Clutton-Brock TH (1991) *The evolution of parental care*. Princeton University Press, Princeton
- Darwin C (1872) *On the origin of species*, sixth edition. 1958 New American Library Mentor, New York
- Day CSD, Galef BG Jr (1977) Pup cannibalism: one aspect of maternal behavior in golden hamsters. *J Comp Physiol Psychol* 91:1179–1189
- Foltz DW (1979) Genetics and mating system of the oldfield mouse (*Peromyscus polionotus*). PhD dissertation, University of Michigan, Ann Arbor
- Foltz DW (1981) Genetic evidence for long-term monogamy in a small rodent, *Peromyscus polionotus*. *Am Nat* 117:665–675
- Gandelman R, Simon NG (1978) Spontaneous pup-killing by mice in response to large litters. *Dev Psychobiol* 11:235–241
- Hosmer DW Jr, Lemeshow S (1989) *Applied logistic regression*. Wiley, New York
- Hrdy SB (1979) Infanticide among mammals: a review, classification, and examination of the implications for reproductive strategies of females. *Ethol Sociobiol* 1:13–40
- Kalbfleisch JD, Prentice RL (1980) *The statistical analysis of failure time data*. Wiley, New York
- Kirkpatrick M, Lande R (1989) The evolution of maternal characters. *Evolution* 43:485–503
- Lacy RC (1992) The effects of inbreeding on isolated populations: are minimum viable population sizes predictable? In: Fiedler PL, Jain SH (eds) *Conservation biology*. Chapman-Hall, New York, pp 277–296
- Lacy RC, Alaks G, Walsh A (1996) Hierarchical analysis of inbreeding depression in *Peromyscus polionotus*. *Evolution* 50:2187–2200
- Layne JN (1968) Ontogeny. In: King JA (ed) *Biology of Peromyscus (Rodentia)*. American Society of Mammalogists, Stillwater, Oklahoma, pp 148–253
- Margulis SW (1996) The effects of inbreeding on parental behavior and reproductive success in two subspecies of the oldfield mouse, *Peromyscus polionotus*. PhD dissertation, University of Chicago
- Margulis SW (1997a) Differential effects of inbreeding at juvenile and adult life history stages in *Peromyscus polionotus*. *J Mammal* (in press)
- Margulis SW (1997b) Relationships among parental inbreeding, parental behavior, and offspring viability in oldfield mice. *Anim Behav* (in press)
- Margulis SW, Altmann J (1997) Behavioral risk factors in the reproductive success of inbred and outbred oldfield mice, *Peromyscus polionotus*. *Anim Behav* (in press)
- Mock DW (1984) Infanticide, siblicide, and avian nestling mortality. In: Hausfater G, Hrdy SB (eds) *Infanticide: comparative and evolutionary perspectives*. Aldine, New York, pp 2–30
- Packer C, Pusey AE (1983a) Male takeovers and female reproductive parameters: a simulation of oestrous synchrony in lions (*Panthera leo*). *Anim Behav* 31:334–340
- Packer C, Pusey AE (1983b) Adaptations of female lions to infanticide by incoming males. *Am Nat* 121:716–728

- Ralls K, Ballou J (1982a) Effects of inbreeding on infant mortality in captive primates. In *J Primatol* 3:491–505
- Ralls K, Ballou J (1982b) Effect of inbreeding on juvenile mortality in some small mammal species. *Lab Anim* 16:159–166
- Ralls K, Brugger F, Ballou J (1979) Inbreeding and juvenile mortality in small populations of ungulates. *Science* 206:1101–1103
- Rood JP (1966) Observations on the reproduction of *Peromyscus* in captivity. *Am Midl Nat* 76:496–503
- SAS (1988a) In: SAS technical report P-179, release 6.03. SAS Institute, Cary
- SAS (1988b) SAS/STAT user's guide, release 6.03. SAS Institute, Cary
- SAS (1990) SAS technical report P-200, release 6.04. SAS Institute, Cary
- Smith MH (1966) The evolutionary significance of certain behavioral, physiological, and morphological adaptations of the old-field mouse, *Peromyscus polionotus*. PhD dissertation, University of Florida, Gainesville
- Smith RH (1979) On selection for inbreeding in polygynous animals. *Heredity* 43:205–211
- Wang Z, Nowak MA (1994) Alloparental care and the influence of father presence on juvenile prairie voles, *Microtus ochrogaster*. *Anim Behav* 47:281–288

Communicated by G. Wilkinson