

Relationships among parental inbreeding, parental behaviour and offspring viability in oldfield mice

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Abstract. Studies of inbreeding depression have traditionally suffered from two weaknesses. First, they usually confound offspring deficiencies with parental ones; second, they neglect the possible role of behaviour in inbreeding depression. In the present study, I examined the relationship among parental inbreeding, offspring viability and parental behaviour in two subspecies of the monogamous oldfield mouse, *Peromyscus polionotus*. Parental inbreeding was separated from any offspring inbreeding effects through both experimental design and analysis. Dams performed more parental behaviour than did sires, and maternal behaviour had a stronger effect on offspring survival than did paternal behaviour. Maternal behaviour was more buffered to the effects of inbreeding than was paternal behaviour; that is, parental behaviour of inbred females was not compromised. In contrast, inbred males showed substantial deficits in parental behaviour, but this did not put their offspring at risk. Although inbred females had lower reproductive success than outbred females, this effect was not manifest in terms of lower offspring viability. Therefore, inbreeding depression manifests itself through deficits on traits of adult females other than maternal care. A possible physiological basis for these findings is hypothesized.

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Mating between close relatives has traditionally been interpreted as detrimental in sexually reproducing species. Darwin (1872, page 274) stated that mating between close relatives ‘... almost always leads to decreased size, weakness, or sterility’, and that inbreeding is generally avoided in nature. The ways in which inbreeding can manifest itself have not received sufficient attention.

Inbreeding depression can manifest itself in at least two ways, on viability and on fertility. Only one of these fitness components, viability, has received extensive attention in non-domesticated animals. The overall costs of inbreeding to juvenile viability have been well studied in laboratory and captive populations (e.g. Sittmann et al. 1966; Ralls et al. 1979; Brewer et al. 1990; Miller & Hedrick 1993). In this context, inbreeding

depression affects fitness because inbred offspring may have a lower probability of survival than outbred offspring. Inbred individuals that survive to breeding age are at least implicitly assumed to be free of the deleterious traits that are considered to indicate inbreeding depression.

If an inbred individual survives to adulthood, inbreeding depression may still arise through effects on fitness components that are expressed later in life (Sharp 1984; Charlesworth & Charlesworth 1987; Margulis & Altmann 1997). An inbred adult may fail to breed, may produce poor-quality offspring (regardless of whether these offspring are themselves inbred) or may fail to raise its offspring (Lacy et al. 1993; Baker et al. 1996; Estep & Dewsbury 1996; Wildt 1996). Rather than attributing offspring viability solely to traits expressed by the offspring, this viability decrement may be due to some behavioural or physiological deficits in the parents.

In the present study, I investigated the relationship between parental behaviour and offspring viability, and the potential differential effects of

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Table I. Experimental design and sample sizes

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

Number of subjects in each experimental group, based on inbreeding coefficient of male, female and litter, if a litter was to be produced. 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).

inbreeding on male and female parental behaviour, in two subspecies of the monogamous oldfield mouse, *Peromyscus polionotus subgriseus* and *P. p. rhoadsi*. Using the same subjects we (Margulis 1996; Margulis & Altmann 1997) demonstrated an effect of inbreeding on reproductive success: pairs in which the female was inbred had reduced fertility (took longer to begin breeding and reared fewer pups during the study) relative to pairs in which the female was outbred, independent of whether the offspring were inbred. Paternal inbreeding, in contrast, did not affect reproductive success.

Offspring viability is the fitness component on which I focused here, and parental behaviour is the trait I examined for its role in viability differences. Because of the paucity of information on parental care in this species, I first examined differences in levels of maternal and paternal care and established the relationship between specific parental behaviours and litter survival. I then examined the effect of inbreeding on parental care. If parental behaviour influences offspring survival, and if inbred parents have lower reproductive success, then one way that this may arise is through inbreeding adversely affecting parental behaviour.

METHODS

Subjects

Subjects were two subspecies of the oldfield mouse. This species is monogamous and both

parents engage in parental behaviour (Smith 1966; Foltz 1979, 1981; D. Gubernick & C. Dold, unpublished data). Descriptions of the wild-caught founder stocks as well as captive housing and maintenance conditions have been described elsewhere (Lacy 1992; Lacy et al. 1996; Margulis & Altmann 1997). Founders were assumed to be unrelated to one another, and both inbred and outbred stocks were maintained in the laboratory. Subjects were seventh- and eighth-generation captive-born. The extensive pedigrees available for the captive population allowed me to establish experimental pairings with known levels of inbreeding.

Experimental Design

The study group consisted of 165 *P. p. 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 I). The pairing scheme allowed me to separate the effects of inbreeding on the parent from inbreeding on the litter, for example by comparing inbred parents that produced inbred litters to outbred parents that produced inbred litters. Animals with inbreeding coefficients greater than 0.1 were considered inbred, although for some analyses, I used inbreeding as a continuous variable. Because the two subspecies did not show significant differences in parental behaviour, I pooled the data.

All subjects were at least 55 days old 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). *Peromyscus polionotus* usually undergoes a postpartum oestrus; thus, females typically produced another litter within a week of the removal of a weaned litter ($\bar{X} \pm SE$ inter-litter interval = 28.8 ± 0.34 days, $N=618$ inter-litter intervals). Pairs were separated 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.

As is common in mammals, parental behaviour and litter viability differed for first versus subsequent litters, with first litters tending to be smaller and having lower viability than subsequent litters (e.g. Wang & Nowak 1994; Margulis, in press). First litters ($N=189$) were therefore excluded from the main behavioural analyses, and their results will be presented separately. The study design also resulted in females rearing their final litter without their mate present. These litters too, were analysed separately, but are used for comparison where appropriate. The final main data set therefore contained data on 352 litters from 207 pairs. Where possible, when data were used from more than one litter per pair, the data were averaged to yield a single value for each pair.

Data Collection

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

Immediate postpartum observations

Peromyscus litters are usually born during daylight hours (Layne 1968). Cages were checked

for the presence of litters each morning. Upon discovery of a litter, I recorded initial litter size and litter number, and conducted a 20-min focal observation (Altmann 1974) of the parents and neonates. During the first 10 min, I recorded the time that each parent spent in contact with the pups and the number of times each parent nuzzled pups. I then added cotton nesting material to the food hopper and collected an additional 10 min of data. All pairs that received cotton built nests by the end of the light period, although not all pairs did so during the observation period. During the second 10 min of observation, I recorded the total time spent nest-building as well as the latency to begin nest-building. Only those litters that contained at least one live pup at the time of the first observation (i.e. the litter had not been completely lost) were included in the analyses ($N=352$ litters).

To obtain a more detailed picture of the behaviour of these nocturnal rodents, I videotaped a subset of 83 pairs (41 *P. p. subgriseus* and 42 *P. p. rhoadsi*). Videotaping allows a more complete description of social interaction by providing a detailed record of all behaviours. Animal maintenance was identical to that in the main study, with the exception of the lighting regime. In addition to the standard room lighting, these pairs were maintained under constant, direct red light (40 W red bulbs, 0.5–1 m from the cages). The direct nature of the lighting, necessary to identify animals on the videotape, meant that the animals experienced more light during the dark, 'active' period than would normally be the case. The lighting regime altered behaviour prior to the birth of any litters and negatively affected reproductive success (Margulis 1996; Margulis & Altmann 1997). However, the behaviour of these pairs following the birth of a litter did not differ significantly in a consistent way from the behaviour of pairs that were not exposed to the direct red lighting. The videotaped pairs were therefore included in the analyses of parental behaviour.

Pairs were videotaped on the day of a litter birth for 24 h, using a time-lapse VCR (Panasonic model #AG6040). I videotaped 105 litters from 46 pairs. Tapes were then viewed and behaviours of interest were scored using an observational software program (P. Margulis, unpublished software). Preliminary videotaping indicated that behaviour during the daylight hours did not vary among subjects, but that during the nocturnal

(i.e. red light only) period did. I analysed only behaviours recorded during the 12-h dark period. The proportion of time during the dark period that subjects spent in each behaviour, as well as frequencies of specific social and sexual behaviours, were recorded (see ethogram in Margulis 1996). The time spent in the nest, in contact with the pup, and inactive were highly correlated; therefore I present only results for the time in the nest. I analysed differences in behaviour as a function of maternal and paternal inbreeding, and litter survival as a function of parental behaviour.

Nest assessments

Preliminary observations suggested that nest quality, indicated by height of nest and proportion of cage floor covered, was a good indicator of litter survival. Litters were more likely to survive when nests were high and when less than half the cage floor was covered by nesting material. In an effort to quantify this phenomenon, I evaluated nest quality at intervals throughout the 20 days of parental care. On days 1, 2, 5, 10 and 20 following the birth of a litter, nest height and area were measured to the nearest 0.5 cm from outside the cage, and nest quality was evaluated subjectively as low quality (low nest, covering more than half of the cage floor), or high quality (maximal height, covering less than half of the cage floor). As expected, nest height and area were significantly associated with nest quality (nest height positively, nest area negatively). Only the subjective quality evaluation was a significant predictor of litter survival, and I therefore used it in analyses of nest quality and litter survival and on the effect of parental inbreeding on nest quality. I made nest assessments on 751 nests.

Behaviour throughout the period of parental care

To obtain a general picture of activity throughout the 20-day period of parental care, twice-weekly scans were made on all 207 pairs that produced at least one litter. Behavioural data were collected when the animals were active, during the lights-off period. I conducted scan samples (Altmann 1974) for 1 h, with scans at 15-min intervals. A miner's headlamp fitted with a red filter was used to aid observation. On each scan, I collected three types of data: (1) whether the members of the pair were in contact; (2) whether

one or both pair members were in the nest with the pups (when pups were present); and (3) the behaviour of the two adults (complete ethogram available in Margulis 1996). Prior to analysing data, I ascertained that neither litter age (0–20 days old) nor stage of pregnancy significantly affected female behaviour. The per cent of time spent in the nest, in contact with the pup and inactive were highly correlated ($r=0.91-1.0$). Therefore, I present results only for the per cent of time in the nest.

Data Analysis

Parental behaviour and offspring viability

Litters were likely to survive or die as a unit (of the 357 litters in which pup loss was observed, the entire litter was lost in 237 cases). The overall rate of total litter loss, 27%, was consistent with overall loss rates observed for *P. polionotus* (18–26%) during long-term demographic monitoring in the Brookfield mouse colony (R. Lacy, personal communication). Rood (1966) reported litter loss rates of 37% for *P. polionotus* in a captive colony at Michigan State University. For analysis, litter survival was scored as a dichotomous variable. Litters were considered to survive if more than half the pups in the litter survived to weaning, and each litter was used as a single data point. I used multiple logistic regression (Hosmer & Lemeshow 1989; SAS LOGISTIC procedure, SAS Institute 1990) to assess the relative importance of various parental behaviours for litter survival both during the immediate postpartum period and throughout the 20 days of parental care. I used survival analysis (Kalbfleisch & Prentice 1980; SAS LIFETEST procedure, SAS Institute 1988a) to analyse the time lapsed until onset of nest-building behaviour for surviving ($N=276$) and non-surviving ($N=75$) litters. A Kruskal–Wallis test (SAS NPAR1WAY procedure, SAS Institute 1988b) was used to assess behavioural differences between males and females.

Inbreeding and parental behaviour

I used non-parametric statistics (Kruskal–Wallis test, SAS NPAR1WAY procedure, SAS Institute 1988b) to evaluate the effect of inbreeding on parental behaviour (proportion of time spent in nest, nest-building, frequency of pup-nuzzling). When appropriate, I calculated

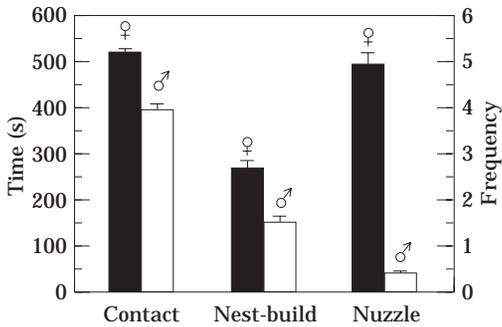


Figure 1. Differences in parental behaviour by male and female oldfield mice, *Peromyscus polionotus*, showing $\bar{X} \pm \text{SE}$ s (of 600) that males and females were in contact with pups and spent nest-building, and number of nuzzles that males and females directed towards pups during 10 min.

means for each pair ($N=207$ pairs that produced litters). I examined the effect of inbreeding on latency to begin nest-building using survival analysis. I analysed the effect of parental inbreeding on nest quality using logistic regression. I examined the frequency of intra-pair sexual behaviour (mounting and naso-anal sniffing; Margulis 1996) from the videotape data, using a Kruskal-Wallis test (SAS NPAR1WAY procedure, SAS Institute 1988b).

RESULTS

Maternal and Paternal Behaviour

Females spent significantly more time in contact with pups than did males, spent more time nest-building and nuzzled pups more frequently than did males during the immediate postpartum period prior to the addition of nesting material (Kruskal-Wallis test, $\chi_1^2=16.97$, $P<0.0001$ for contact; $\chi_1^2=246.60$, $P<0.0001$, for nuzzling; $\chi_1^2=31.03$, $P<0.0001$ for nest-building, $N=207$; Fig. 1). Males also had significantly longer latencies than females to begin nest-building (survival analysis, Wilcoxon $\chi_1^2=54.57$, $N=352$ litters, $P<0.0001$; $\bar{X} \pm \text{SE}$ latency= 381.48 ± 13.14 s for males, 246.89 ± 13.97 s for females).

Males were rarely in the nest and in contact with pups unless the female was also in the nest. Both parents were simultaneously in contact with newborn litters 63.3% of the 10-min postpartum observation; females were alone with the pups 22.9% of this time, and males were alone with the pups for only 3.1% of the time. During the full 20 days of parental care, one or both parents were in the nest with the pups approximately 60% of the time (Fig. 2).

If parents show compensatory behaviour (i.e. the male is present in the nest when the female is

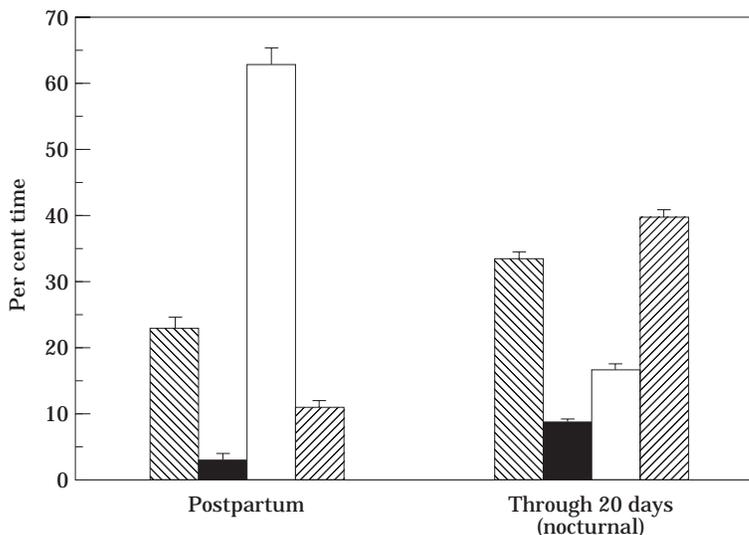


Figure 2. Per cent of time that parents spent in contact with pups during the immediate postpartum period (left) and in the nest with pups, as measured by twice-weekly scans throughout the 20-day period of parental care (right). ▨: Female only; ■: male only; □: both parents; ▩: pup alone.

absent and vice versa), a negative relationship would be expected between the time the male and female remained in the nest with pups. On the other hand, because males were rarely in the nest without the female, we might instead expect male behaviour to track that of his mate, resulting in a positive relationship between male and female behaviour. The time that males spent in the nest tracked that of their mate (linear regression, $Y=0.87X-51.80$, where Y =male time on nest and X =female time on nest, $r^2=0.32$, $F_{1,205}=98.23$, $P<0.0001$). No compensatory behaviour was evident. Male latency to begin nest-building increased as female latency increased (survival analysis, $\chi^2_1=113.2$, $N=352$ litters, $P<0.0001$).

Parental Behaviour and Offspring Viability

The probability of litter survival increased during the immediate postpartum period as both maternal and paternal behaviour increased during the postpartum period, based on a composite parental behaviour score for each male and for each female (time spent in contact with pups + time spent nest-building; logistic regression, Wald $\chi^2_1=8.25$, $P<0.004$ for females, $\chi^2_1=7.11$, $P<0.008$ for males). For the full 20-day period of parental care, however, only maternal behaviour increased the probability of litter survival (Wald $\chi^2_1=16.87$, $P<0.0001$; paternal behaviour: $\chi^2_1=1.96$, $P<0.16$; Fig. 3). Figure 4 shows the relationship between parental care and litter survival, based on the logistic regression analyses. During the immediate postpartum period, female behaviour contributed only slightly more towards differences in litter survival than did male behaviour. However, the steepness of the response surface was substantially greater for the female than for the male for behaviour throughout the 20-day period, indicating that male behaviour contributed little to differences in litter survival after the immediate postpartum period. For first litters, only female behaviour predicted litter survival (Wald $\chi^2_1=8.05$, $P<0.005$; for males, $\chi^2_1=1.91$, $P<0.17$).

Because male behaviour was so dependent on female behaviour, I repeated the analyses on the component behaviours (contact with pups and nest-building) omitting male behaviour from the logistic regression models. Litters were significantly more likely to survive when females

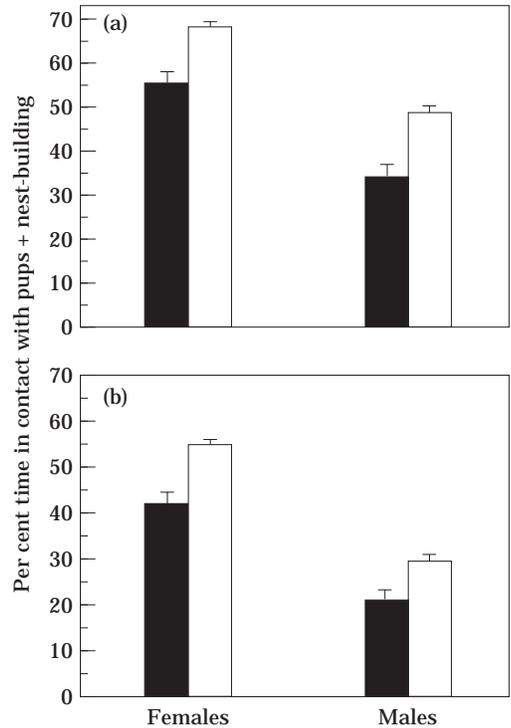


Figure 3. Composite parental behaviour scores for parents of non-surviving (■) versus surviving (□) litters. Scores represent the per cent of time parents spent in contact with pups plus the per cent time spent nest-building during (a) the postpartum period and (b) throughout the period of parental care.

spent more time in contact with pups during the immediate postpartum period (Wald $\chi^2_1=9.77$, $P<0.0018$), and in the nest with pups throughout the parental care period ($\chi^2_1=6.12$, $P<0.013$). The probability of litter survival increased with the time that females engaged in nest-building ($\chi^2_1=7.73$, $P<0.005$). Litters were more likely to survive as the latency to begin nest-building declined (survival analysis, Wilcoxon $\chi^2_1=6.53$, $P<0.01$ for females, $\chi^2_1=6.29$, $P<0.01$ for males).

The nest quality assessments showed that litters were more likely to survive as nest quality increased (logistic regression, $\chi^2_1=24.91$, $P<0.0001$ on day 1, $\chi^2_1=7.61$, $P<0.006$ on day 20). Nest quality did not differ for first versus subsequent litters.

Parental Inbreeding and Parental Behaviour

During the immediate postpartum period, inbred and outbred females did not differ either

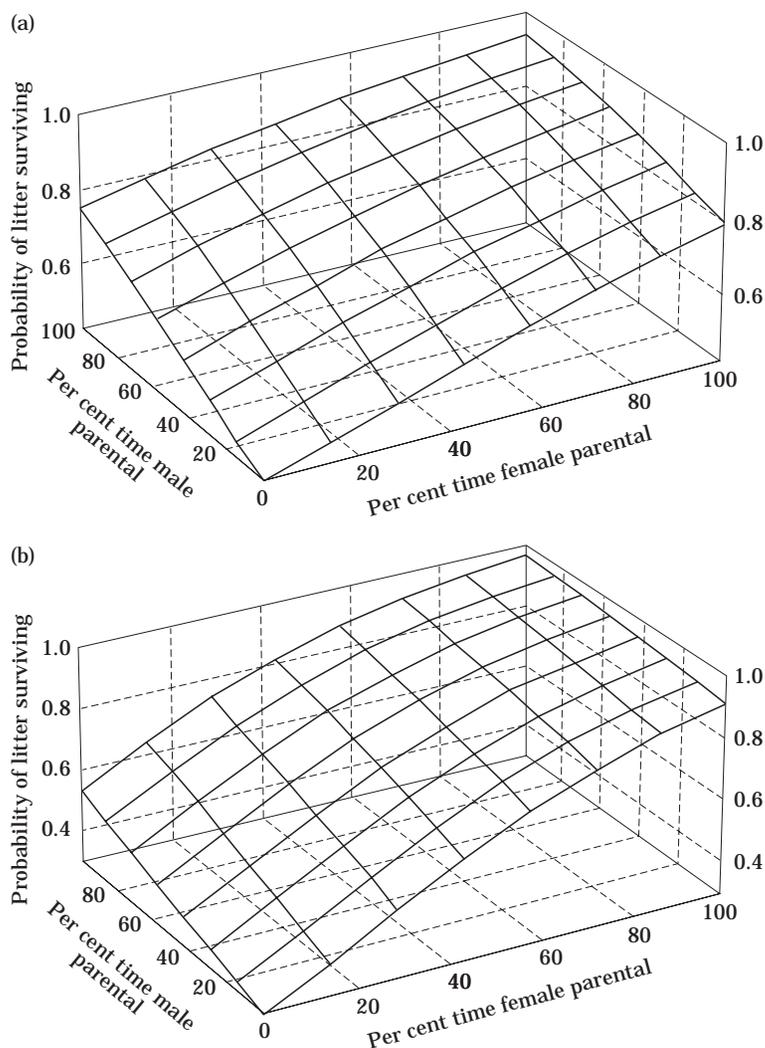


Figure 4. Predicted probability of survival based on parental behaviour during (a) the postpartum period and (b) throughout the 20 days of parental care. The comparative steepness of the response surface in each plane indicates the relative importance of male and female parental behaviour for litter survival.

for first litters or subsequent litters in time spent in nest with pups (Kruskal–Wallis test, first litters: $\chi_1^2=0.08$, $P<0.77$, later litters: $\chi_1^2=1.94$, $P<0.16$), or time spent nest-building (first litters: $\chi_1^2=0.002$, $P<0.96$, later litters: $\chi_1^2=0.32$, $P<0.58$; Fig. 5). Inbred females nuzzled pups significantly more often for first but not subsequent litters ($\chi_1^2=7.49$, $P<0.006$ and $\chi_1^2=0.04$, $P<0.86$, respectively). In contrast, outbred males spent significantly more time nest-building than did inbred males for later litters, but not for first litters (Kruskal–Wallis test,

first litters: $\chi_1^2=0.03$, $P<0.86$, later litters: $\chi_1^2=5.17$, $P<0.02$), and more time in contact with pups for first litters, but not for later litters (first litters: $\chi_1^2=4.76$, $P<0.03$, later litters: $\chi_1^2=1.59$, $P<0.21$). Inbred and outbred males did not differ in frequency of pup-nuzzling for first or subsequent litters ($\chi_1^2=0.39$, $P<0.53$ and $\chi_1^2=1.11$, $P<0.29$, respectively).

As maternal inbreeding increased, females began nest-building more quickly (survival analysis, Wilcoxon $\chi_1^2=5.35$, $P<0.02$), but inbred males

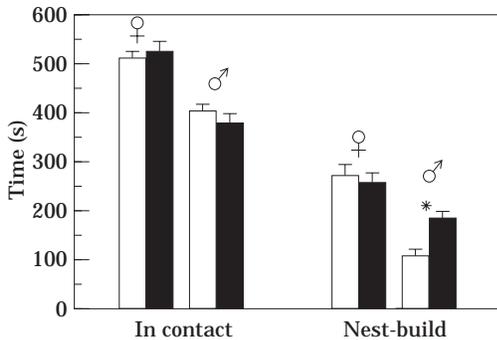


Figure 5. The effect of parental inbreeding on parental behaviour during the postpartum period. Seconds (of 600) spent in contact with pups (left), and nest-building (right) for inbred (□) and outbred (■) males and females. * $P < 0.05$.

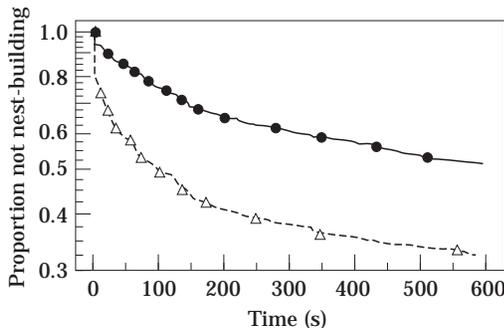


Figure 6. Survivorship curve indicating the latency to begin nest-building for males (●) and females (△). The curves indicate the proportion of subjects that had not yet begun nest-building by 600 s. Male latency increased as male inbreeding coefficient increased ($\chi^2_1 = 13.85$, $P < 0.002$). In contrast, female latency decreased as female inbreeding coefficient increased ($\chi^2_1 = 5.35$, $P < 0.02$).

were significantly slower to begin nest-building ($\chi^2_1 = 13.85$, $P < 0.0002$; Fig. 6). Because of the nature of survival analysis, each litter was used as a data point when analysing latency to begin nest-building ($N = 352$ litters). Inbreeding did not affect latency to begin nest-building for first litters, although for both males and females, latencies were significantly greater for first litters than for subsequent ones (females: $\chi^2_1 = 46.88$, $P < 0.0001$, $\bar{X} \pm SE = 398.6 \pm 17.6$ s for first litters, 246.9 ± 14.0 s for later litters; males: $\chi^2_1 = 10.68$, $P < 0.001$, 447.7 ± 16.7 s for first litters, 381.5 ± 13.1 s for later litters). No differences in

time-in-nest were detected for inbred and outbred subjects from the videotape data, nor did inbred and outbred video subjects differ in frequency of specific socio-sexual behaviours (nasal-anal sniffing, mounting).

Inbred females tended to spend more time in the nest with pups than did outbred females throughout the 20 days of parental care (Kruskal-Wallis test, $\chi^2_1 = 2.75$, $P < 0.097$), but inbred males tended to spend less time in the nest than did outbred males ($\chi^2_1 = 2.53$, $P < 0.11$). However, inbred females spent less time nest-building during the 20-day period than did outbred females ($\chi^2_1 = 4.03$, $P < 0.04$). Most nest-building took place on the day of parturition (females were observed nest-building in only 1% of the nocturnal scans throughout the 20 day pre-weaning period).

Neither maternal inbreeding nor paternal inbreeding affected nest quality in multiparous pairs. For primiparous pairs, however, nest quality assessed on or after day 5 was lower when sires were inbred (logistic regression, $\chi^2_1 = 11.12$, $P < 0.0009$ on day 5; $\chi^2_1 = 5.13$, $P < 0.024$ on day 10; $\chi^2_1 = 4.26$, $P < 0.04$ on day 20).

DISCUSSION

Male and female parental behaviour differed greatly in their effect on litter survival, and were affected differently by parental inbreeding. Maternal behaviour positively influenced litter survival, and was not compromised when the dam was inbred. In contrast, paternal behaviour was negatively affected when the sire was inbred, but did not impose a survival cost on the litter. Maternal behaviour, with its stronger role in determining litter survival, appears to be buffered more than paternal behaviour, to the possible detrimental effects of inbreeding.

The relative contribution of male and female parents to litter survival varies considerably among rodents. In some monogamous rodents, a female decreases levels of parental care as the male increases his level of parental care (*Acomys cahirinus*: Makin & Porter 1984; *Microtus ochrogaster*: Wang & Nowak 1992; *P. eremicus*: Hatton & Meyer 1973; *P. californicus*: Dudley 1974b). In other species, the male's behaviour tends to match or track that of his mate (*P. californicus*: Gubernick & Alberts 1987, 1989; *P. polionotus*: Smith 1966; Foltz 1979).

Even in monogamous species, the degree to which mother and father engage in parental care varies, as does their effects on each other and on litter survival. In some species male care is approximately equal to that of the female, but in others, the male provides significantly less care than does the female. In laboratory studies of *P. californicus*, for example, fathers were observed to engage in all forms of parental behaviour (except lactation), and did so to the same degree as did mothers (Dudley 1974b; Gubernick & Alberts 1987, 1989). In contrast, female *A. cahirinus* (Makin & Porter 1984) performed significantly more parental care than did males in the laboratory; the same was true for *M. pinetorum* (FitzGerald & Madison 1983; Oliveras & Nowak 1986). In the present study, *P. polionotus subgriseus* and *P. p. rhoadsi* males performed significantly less parental care than females. In contrast, D. Gubernick & C. Dold (unpublished data) found no differences in levels of parental behaviour performed by male and female oldfield mice, *P. polionotus leucocephalus*. However, they used proven breeders, they did not specifically examine effects of behaviour on litter survival and focused on behaviour beginning after the period during which most litter mortality occurs (Layne 1968).

The thermoregulatory role of nest-building makes this a natural behaviour to examine with respect to litter survival (Lynch 1977). Nest size on the day of parturition was positively correlated with litter survival in *Mus musculus* (Lynch & Possidente 1978). The present findings, that increased time spent nest-building and decreased latency to begin nest-building, were positively associated with litter survival, are consistent with expectation.

Parental presence in the nest serves a thermoregulatory function, and male presence may improve pup survivorship and developmental rate in the laboratory (*P. californicus*: Dudley 1974a; *M. ochrogaster*: Wang & Nowak 1992; *Phodopus campbelli*: Wynne-Edwards 1987; Wynne-Edwards & Lisk 1989), although possibly only under ecologically relevant conditions (cold challenge and foraging for food; Gubernick et al. 1993). In the present study, *P. polionotus* litters showed no increase in survival when the males were present (when compared to last litters, which were reared by the female only). The conditions under which I conducted the present study did not

impose those environmental stressors that are typically believed to contribute to differences in litter survival in the wild. If the male's presence were to have a substantial effect on litter survival, it might be most readily observed under environmentally relevant conditions, as has been shown for *P. californicus* (Gubernick et al. 1993).

The contribution of male *P. polionotus* to successful rearing of a litter may be less direct. Unlike direct parental care (e.g. feeding pups), which has immediate fitness consequences for offspring, indirect investment need not involve parent-offspring interactions specifically, but may contribute to offspring survival none the less. Such indirect behaviours include territory or burrow acquisition and defence, predator defence and protection against conspecific intruders (Kleiman & Malcolm 1981). I did not examine male indirect investment in the present study.

Unlike some rodent species in which monogamy and biparental care have been documented, I found no evidence in oldfield mice of compensatory behaviour by parents. Although female prairie voles, *M. ochrogaster*, decreased the time they spent in the nest with pups as the males' time in the nest increased (Wang & Novak 1992), female *P. polionotus* did not. To the contrary, I suggest that males in the present study were reacting to, or 'matching' the behaviour of their mates (since males that spent the most time in contact with their litters had mates that spent more time with their litters, as well). Males may accrue thermoregulatory benefits by remaining in the nest with their mates, who coincidentally are in contact with the litter. Females may benefit similarly. In *P. californicus*, male parental behaviour is maintained by the presence of the female, but female parental behaviour is independent of the male's presence (Gubernick & Alberts 1989). The present results suggest that this may be the case in *P. polionotus* as well, and support the finding that males may in fact 'match' their behaviour to that of their mates.

Given that inbred adult females produce fewer surviving offspring than do outbred females (Margulis 1996; Margulis & Altmann 1997), and the present findings that parental behaviour (maternal behaviour in particular) contributes to litter survival, we might predict that the decreased reproductive success is brought about at least partially by a detrimental effect of inbreeding on parental behaviour.

Inbreeding had different effects on male and female parental behaviour. Inbred females either showed improved parental behaviours (shorter latency to begin nest-building, more frequent nuzzling) or did not differ from outbred females. The only exception to this pattern was the per cent of time spent nest-building during the 20-day period of parental care, a fairly rare behaviour. Males, on the other hand, tended to show a decline in parental behaviours if they were inbred. They showed a more consistent response to inbreeding, and in no case did inbred males out-perform outbred males.

Inbreeding thus did not have detrimental effects on maternal behaviour, but it did result in impaired paternal behaviour. Because male parental behaviour did not significantly affect litter survival, these results suggest that inbreeding does not negatively affect reproductive success. Margulis (1996, *in press*) showed that litters born to inbred females were somewhat more likely to survive than were the litters of outbred females, consistent with the present findings that maternal behaviour is not impaired, and may show improvement, when mothers are inbred. Inbred females nevertheless show reduced reproductive success compared to outbred females, including reduced fertility (as indicated by a significant delay in the onset of breeding), and reduced activity levels (Margulis 1996; Margulis & Altmann 1997). Although inbreeding does not affect maternal behaviour, it does affect traits that are expressed in adult females and results in reduced reproductive success.

In a monogamous species, the variance in reproductive success of females will equal that of males, provided that paternity certainty is high (as it appears to be for *P. polionotus*; Foltz 1981). Considering the monogamous pair as a reproductive unit, pairs in which the female is inbred will ultimately rear fewer young than will pairs in which the female is outbred, even when progeny are not inbred. Thus, if we consider the effect of inbreeding on the population as a whole, inbreeding depression is occurring, and it is manifested in the adult animal.

The way in which inbreeding affects female fertility and parental behaviour may be under the control of different mechanisms. However, I speculate that a single underlying phenomenon may contribute to these two disparate findings. Preliminary data (K. Ryan, L. Feddersen & S.

Margulis, unpublished data) suggest that inbred females were more likely to experience pseudo-pregnancy, which is generally associated with extended luteal function and therefore increased levels of progesterone (Kenney et al. 1977). Progesterone administration results in increased nest-building activity in strains of *Mus musculus* that had been selected for low nest-building scores (Schneider et al. 1983). Elevated progesterone is also associated with decreased activity level in a number of rodent species (Takahashi & Menaker 1980; Labyak & Lee 1995). Females that failed to breed were significantly less active than females that bred, and in one subspecies, inbred females were significantly less active than outbred females (Margulis 1996; Margulis & Altmann 1997). If inbreeding were to result in changes in progesterone secretion or sensitivity, the result could be less-regular cycling (reduced fertility) combined with decreased activity and increased nest-building behaviour (improved parental behaviour). This possibility awaits further investigation.

These results suggest that the traditional method of measuring inbreeding depression as juvenile mortality may underestimate the extent of inbreeding depression in a population. Because inbreeding depression may influence different fitness components in varying ways, and may affect different traits due to chance processes (Lacy et al. 1996; Margulis 1996; Margulis & Altmann 1997), a careful analysis of the way in which inbreeding influences reproductive behaviour and physiology is essential to assess accurately the impact of inbreeding on captive populations and small, remnant wild populations, in which inbreeding may be unavoidable.

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