Behavioural risk factors in the reproduction of inbred and outbred oldfield mice

SUSAN W. MARGULIS*‡ & JEANNE ALTMANN*†‡

**Committee on Evolutionary Biology and †Department of Ecology and Evolution,
The University of Chicago,
‡Department of Conservation Biology, Brookfield Zoo*

Abstract. The present study investigated two rarely measured aspects of inbreeding depression: the relationship between inbreeding and behaviour, and the possibility that inbred individuals that survive infancy may still suffer from inbreeding depression by failing to breed or failing to show appropriate mating or parental behaviours. Specifically, the relationship between (1) behaviour at pairing and reproductive success, (2) inbreeding and reproductive success and (3) inbreeding and pairing behaviour, was examined in two subspecies of the oldfield mouse, *Peromyscus polionotus*. Effects of parental and offspring inbreeding were separated through experimental design and analysis. Activity level during the first 25 days after pairing predicted future reproductive success: pairs that remained less active during the nocturnal (active) period were significantly less likely to breed than pairs that remained more active. Inbred females took significantly longer to produce their first litters and were less likely to produce litters than were outbred females, independently of whether females were related to their mates (i.e. whether their offspring would be inbred). Inactive pairs averaged fewer surviving pups than did active pairs. Inbreeding coefficient of female was a significant predictor of activity level in one of the two subspecies, suggesting that inbreeding may affect behaviour. Inbred adult females showed inbreeding depression in the form of lower conception rates and fewer surviving offspring, although the specific traits affected differed for the two subspecies. The implications for captive breeding programs, and likely causes of the subspecific differences, are discussed.   © 1997 The Association for the Study of Animal Behaviour

A complex suite of behaviours is critical for successful reproduction. In sexually reproducing species, particularly those with biparental care, success depends both on individual traits of several parties (father, mother and offspring) and on their interactions. Not only do parent–offspring interactions influence offspring survival, but so too do intra-pair interactions, particularly in species in which both parents contribute to offspring care. Paired animals do not always breed, and if they do breed, they do not necessarily raise their young. That reproductive success is a function of parental phenotypes and offspring phenotypes has recently been incorporated into population genetics models (Kirkpatrick & Lande 1989), an area in which research has typically focused solely on a single generation.

Reproductive failure may have behavioural as well as physical causes. Inbreeding depression, defined as a reduction in the mean phenotypic value of a character brought about by inbreeding (Falconer 1989), has typically been viewed as a phenomenon that affects physical characters such as birth weight and developmental anomalies. As a result, long-term consequences of inbreeding in mammals have most often been measured only at early stages in an organism's life history, particularly in the form of neonatal survival. The seminal studies by Ralls and colleagues (Ralls et al. 1979; Ballou & Ralls 1982; Ralls & Ballou 1982a, b), for example, although documenting the widespread occurrence of inbreeding depression in captive

Correspondence: S. W. Margulis, Committee on Evolutionary Biology, 940 E. 57th Street, The University of Chicago, Chicago, IL 60637, U.S.A. (email: swm4@ midway.uchicago.edu).

mammalian populations, focused solely on juvenile mortality as the measure of inbreeding depression. In some studies, fertility has ostensibly been analysed, but usually by measuring the number of surviving offspring, thus confounding parental and offspring traits (Brewer et al. 1990; cf. Packer & Pusey 1993).

Not only have most of these studies examined the extent of inbreeding depression solely at early life history stages, but they have examined it only from the perspective of the offspring: do inbred offspring have higher neonatal mortality, or do parents show reduced reproductive success because they are related to one another, and their offspring are therefore inbred and are thus deficient in some way? In such cases, it has been impossible to determine whether poor offspring survival and low parental reproductive success could be attributed to some inherent abnormality in the inbred offspring, or to a behavioural deficit in the parents, because inbreeding of offspring and of parents have commonly been confounded. That is, in those cases where offspring were inbred, the parents were usually inbred as well, or the level of inbreeding of the parents was not controlled for. The possibility that parental behaviour may influence offspring production and survival has not been thoroughly investigated in studies of inbreeding depression (but see Lynch 1977; Lynch & Possidente 1978).

As part of an intensive study on the effects of inbreeding on parental behaviour in the monogamous, nocturnal oldfield mouse, *Peromyscus polionotus*, we also investigated those aspects of reproductive success that precede parental behaviour: pair-affiliative behaviour and time to first reproduction. We examined the effects of inbreeding much later in an organism's life than infancy: after sexual maturity, when reproduction is attempted.

We tested the predictions that the behaviour of pairs that fail to reproduce will differ from the behaviour of pairs that ultimately breed, and that inbred parents will ultimately have lower reproductive success than outbred parents, regardless of the level of inbreeding of their litters. If so, then inbreeding may affect traits and components of fitness that are not manifested until adulthood. We tested these predictions by addressing three questions: (1) whether specific behaviour patterns are predictive of future reproductive success, (2) whether parental inbreeding predicts reproductive success, and (3) whether parental inbreeding influences relevant pair-affiliative behaviour.

METHODS

Subjects

We used three sets of study groups. The main study group consisted of two subspecies of the oldfield mouse, *Peromyscus polionotus*. This species is nocturnal, and convincing field evidence for monogamy exists (Foltz 1979, 1981). *Peromyscus polionotus subgriseus* were captured from the Ocala National Forest in north-central Florida in 1990. *Peromyscus polionotus rhoadsi* were captured near Lake Placid, Florida, in 1990 and 1991. The second data set which was used for a pilot study, comprised animals from another subspecies, *P. polionotus leucocephalus*. Only a few *P. p. leucocephalus* were used, and they bred so poorly that they were not included in the main study. The outcome of this small study was relevant to the results of the main study, however, and are discussed briefly. Stocks for these subjects were captured in the summers of 1990 and 1991 on Santa Rosa Island, Florida (Lacy et al. 1996). Finally, a third data set derives from additional *P. p. subgriseus* and *P. p. rhoadsi* subjects that were videotaped to provide more detailed behavioural data. These study groups were exposed to constant, direct red lighting. All results refer to the main study group, unless otherwise noted. Captive stocks of all three subspecies 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). The extensive pedigrees available for the captive population allowed us to establish experimental pairings with known levels of inbreeding.

Experimental Design and Data Collection

Main and pilot studies

We established 124 *P. polionotus subgriseus* pairs, 125 *P. polionotus rhoadsi* pairs and 48 *P. p. leucocephalus* pairs, divided into eight experimental groups based on maternal inbreeding, paternal inbreeding and the inbreeding coefficient of potential future litters (Table I). The pairing scheme allowed us to separate the effects of inbreeding on

Table I. Experimental design and sample sizes. *Leucocephalus* used for pilot study only

| Maternal f | Paternal f | Litter f | Experimental groups | | |
			subgriseus N	*rhoadsi* N	*leucocephalus* N
i	i	i	16 (5)	16 (6)	6
i	i	o	14 (6)	17 (4)	6
o	o	i	15 (6)	15 (5)	6
o	o	o	16 (5)	17 (5)	6
o	i	i	16 (4)	16 (5)	6
o	i	o	15 (5)	14 (6)	6
i	o	i	16 (5)	15 (6)	6
i	o	o	16 (5)	15 (5)	6
			124 (41)	125 (42)	48

i=Inbred ($f>0.1$; range=0.1010–0.5859), o=outbred ($f\leq0.1$; range=0–0.0980). Numbers in parentheses indicate the additional animals used for the videotape study.

the parent from inbreeding on the litter, for example, by comparing inbred parents that produced inbred litters to outbred parents that produced inbred litters. If inbreeding depression is a result of a deficit in the offspring, then these two groups should not differ, because offspring are inbred in both cases. If inbreeding has a detrimental effect on adults, however, then the group with inbred parents should show a more severe inbreeding depression than do outbred parents. For example, a pair in which the parents were inbred and their litter was outbred might be formed by pairing a male and female that were the products of full-sibling matings, but were not related to one another. A pair in which parents were outbred and their litter was inbred might be formed by setting up a mating between full or half siblings. In no case were litter-mates paired. Animals with inbreeding coefficients greater than 0.1 were considered inbred. We chose the cut-off of 0.1 for several reasons. Low levels of inbreeding are impossible to avoid even in a large, outbreeding population. The inclusion of animals with very low inbreeding coefficients in the inbred category, as has often been done in the past (Ralls et al. 1979; Ballou & Ralls 1982; Ralls & Ballou 1982a, b), also confounds the inbreeding effect with the captivity effect. For some analyses, inbreeding was used as a continuous variable. Inbred *rhoadsi* subjects tended to have slightly higher inbreeding coefficients than did inbred *subgriseus* subjects ($\bar{X}\pm$ SE=0.21 ± 0.01 for *subgriseus* males, 0.23 ± 0.01 for *rhoadsi* males; 0.20 ± 0.01 for *subgriseus* females, 0.27 ± 0.01 for *rhoadsi* females).

Pairs were housed in standard polycarbonate mouse cages, and received mouse chow and water ad libitum. They were maintained on a 12:12 h light:dark cycle. All subjects were at least 55 days of age at the time of pairing. Pairs were kept together through the birth and rearing of three litters, or for 120 days, whichever came sooner. *Peromyscus polionotus* undergoes a postpartum oestrus. Gestation length is approximately 23–24 days (Smith 1966). In the present study, no litter was born prior to the 25th day after pairing. Therefore, we focused on behavioural data gathered during the first 25 days after pairing, before the birth of any litters. Females may have been pregnant during part of this period. Pregnancy did not significantly affect the behaviour of females (Margulis 1996). Measures of demographic outcome were collected throughout the 120 days of the study.

Because *Peromyscus* are nocturnal, behavioural data were collected when the animals were active, during the lights-off period, twice a week. All behavioural observations were conducted without knowledge of inbreeding level of subjects or the relationship of pair members to one another. Scan samples (Altmann 1974) were carried out 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, we collected two types of data: first, whether the members of the pair were in contact, and, secondly, the behaviour of the two animals (see Margulis 1996 for complete ethogram). Raw data were converted into per cent time each subject spent engaging in each

behaviour, or per cent time in contact with mate. Allogrooming, nuzzling and fighting occurred so infrequently that they could not be analysed statistically. Results are presented here for level of inactivity; we defined inactive as a subject sitting immobile, with eyes open or closed. A Psion palmtop computer was used to gather the data. To facilitate identification, several days before pairing, one member of each pair was dyed with black hair dye (Clairol Nice 'n Easy Hair Dye). We 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).

Video study

To obtain a more detailed picture of the behaviour of these nocturnal rodents, we videotaped an additional 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 during a 24-h period (Margulis 1996). These pairs were evenly distributed across the same eight experimental conditions as for the main study (Table I). 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 watt bulbs, positioned 0.5–1.0 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. Because all videotaped pairs were subjected to the same lighting regime, however, differences due to inbreeding should be informative. The 'video' pairs were taped for blocks of up to 24 h at a time using a time-lapse VCR (Panasonic model no. AG6040). Pairs were taped on the day of pairing, and on days 4 and 7 after pairing. Tapes were then scored using an observational software program (P. Margulis, unpublished software), and we recorded the proportion of the dark period (i.e. red lights only) that subjects spent in contact with their mates, inactive but not in contact with mate, active, and grooming their mates, as well as frequencies of specific social and sexual behaviours. We analysed differences in behaviour as a function of maternal inbreeding, paternal inbreeding and inbreeding of potential litters. Subjects of

the video study were also included in the nocturnal scans, although we analysed these data separately due to the observed effect of lighting on behaviour and reproduction.

Data Analysis

Behaviour and reproductive success

The behavioural data were not normally distributed, so we used non-parametric statistics to analyse these data. In most cases, Savage scores, suitable when data are exponentially distributed, were used (SAS NPAR1WAY procedure, SAS Institute 1988b). Logistic regression (Hosmer & Lemeshow 1989) was used to assess the relationship between behaviour and reproductive success when the dependent variable was dichotomous (SAS LOGISTIC procedure, SAS Institute 1990). Animals were therefore categorized as either eventual breeders or non-breeders for this analysis.

Inbreeding and reproductive success

Survival analysis was used to evaluate time to first reproduction (SAS LIFETEST procedure, SAS Institute 1988a) based on inbreeding level of male, female and litter (if produced). Survival analysis is applicable to failure-time data, such as is typically found in biomedical research (Kalbfleisch & Prentice 1980). The endpoint of failure-time data is time until an event occurs. In biomedical research, for example, how long does a specific drug treatment prolong life in study groups? 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 analyses allow these incomplete values to be included in the analyses as 'censored' data. In the present study, the endpoint in question is birth of a litter. Censored data arose from pairs that had not reproduced by 120 days (main study: $N=21$ *subgriseus*, 12 *rhoadsi*; video study: $N=13$ *subgriseus*, 10 *rhoadsi*), that had died, or that had been removed from the study before 120 days due to serious injury (main study: $N=3$ *subgriseus*, 9 *rhoadsi*; video study: $N=4$ *subgriseus*, 1 *rhoadsi*).

Inbreeding and behaviour

Logistic regression (Hosmer & Lemeshow 1989) was used to assess the relationship between

Table II. Comparison of selected results for pairs in the videotape and main (non-video) studies

	P. p. subgriseus		*P. p. rhoadsi*	
	Video	Non-video	Video	Non-video
Median time to first litter (days)	73	34	47.5	30
% Not breeding	35.9	19.7	28.6	17.6
% Time inactive during nocturnal period	18.8	10.7	23	12.2

inbreeding and behaviour, when the dependent behavioural variable was dichotomous. Animals were categorized as 'active' if they spent less than 40% of the nocturnal period inactive and 'inactive' if they spent more than 40% of the nocturnal period inactive. The 40% cutoff is approximately 2 standard deviations above the mean per cent time inactive for both subspecies. The SAS LOGISTIC procedure (SAS Institute 1990) was used to carry out the logistic regression analyses.

RESULTS

Behaviour and Reproductive Success

Pairs in which the female was more inactive in the first 25 days (prior to the birth of any litters) were significantly less likely to breed at all in both the *subgriseus* and *rhoadsi* subspecies (Savage test: $\chi_1^2 = 14.08$, $P < 0.0002$ for *subgriseus*; $\chi_1^2 = 5.47$, $P < 0.02$ for *rhoadsi*). *Leucocephalus* and videotaped pairs extended the range of inactivity that could be investigated. *Leucocephalus* females were inactive $41.59 \pm 3.4\%$ ($\bar{X} \pm$ SE) of the active period, and only three of 48 pairs reproduced. Similarly, the females of the video study were less active, and less likely to breed, than were the females of the main study (Table II). Male activity did not significantly affect reproductive success.

The probability of reproductive failure increased as female's level of inactivity increased (logistic regression, Wald: $\chi_1^2 = 21.96$, $P < 0.0001$). The regression line in Fig. 1 presents the predicted proportion of pairs that should fail to breed for a given female inactivity level, based on a logistic regression analysis on the main study subjects (pooled for the two subspecies). The two subspecies did not differ in mean level of inactivity ($\bar{X} \pm$ SE $= 10.65 \pm 1.3\%$ of the nocturnal period for *P. p. subgriseus*, $12.20 \pm 2.3\%$ for *P. p. rhoadsi*). The actual proportion of pairs failing to breed and
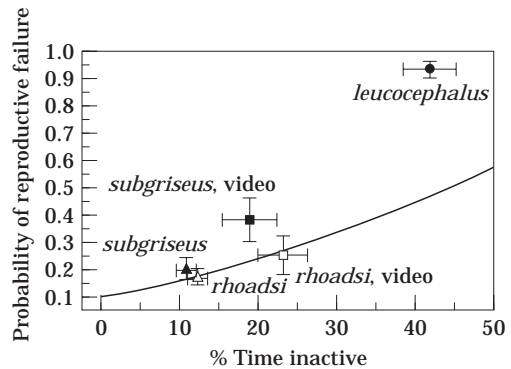

Figure 1. Predicted and observed proportion of pairs failing to breed based on level of inactivity. The curve indicates the predicted values, based on the logistic regression analysis. Actual values, with standard error bars, for each of the three subspecies, and the videotaped subjects, are indicated by points on the graph.

mean level of inactivity for the two main study subspecies, the two subspecies from the videotape study and the *P. p. leucocephalus* pilot study, are indicated on the graph. Both the *subgriseus* and *rhoadsi* groups fell close to the predictive line based on this pooled sample, as did the videotaped *rhoadsi* subjects. The videotaped *subgriseus* subjects fell well above the predictive line, however, as did the *leucocephalus* group. The level of reproductive failure associated with high levels of inactivity was even greater than anticipated from the more restricted range of values observed in the main study.

For the *subgriseus* subspecies, pairs that spent more time in contact with their mates prior to the birth of any litters were significantly less likely to reproduce at all (Savage test: $\chi_1^2 = 5.42$, $P < 0.02$). This difference was not significant in the *rhoadsi* subspecies.

Subgriseus pairs in which the female was inactive averaged fewer pups surviving to twenty
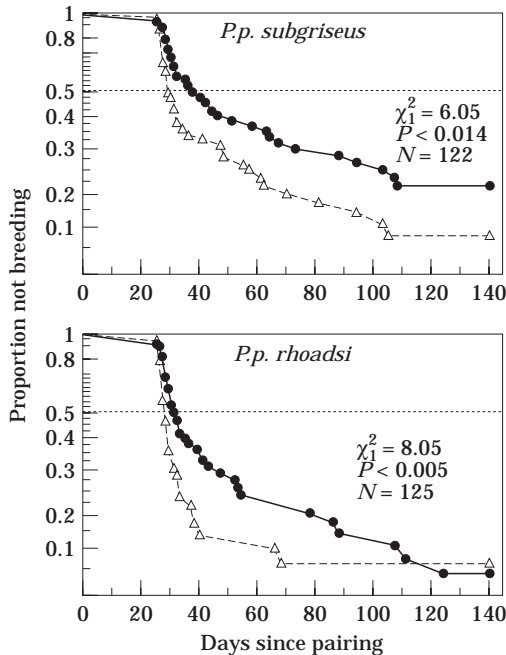
Figure 2. Survivorship curves for time to first litter birth, inbred (●) and outbred (△) females. Data for *subgriseus* and *rhoadsi* populations are plotted separately. The dotted line indicates the median. Curves illustrate the proportion of pairs that had not yet given birth to a litter (*Y* axis) by a given number of days after pairing (*X* axis). Pairs were separated at 120 days if they had not yet produced a litter.

days of age than did pairs in which the female was active (one-tailed $t_1 = 1.91$, $P < 0.05$). The difference was not significant for *rhoadsi* pairs ($t_1 = 1.03$, $P > 0.1$). However, female inactivity significantly decreased the probability of a pair breeding in both subspecies (Margulis 1996).

Inbreeding and Reproductive Success

Inbred females took significantly longer than outbred females to produce their first litters for the two subspecies pooled (survival analysis, Wilcoxon test: $\chi_1^2 = 13.26$, $P < 0.0003$). The two subspecies differed significantly in details of their responses to inbreeding, however ($\chi_1^2 = 6.5$, $P < 0.01$), and were therefore analysed separately. The survivorship curves in Fig. 2 present days from pairing to production of first litter separately for the two subspecies. The initial delay seen in all curves represents the necessary ges-

tational lag from pairing until the first litters are born. The slope of the curves indicates the probability of producing a litter in that time period, with a steep slope representing a greater probability, and a shallower slope a lower probability of producing a litter. The point at which the curves flatten out represents the proportion of pairs that failed to breed. The median time to first reproduction differed considerably for inbred versus outbred dams: 50% of *subgriseus* pairs with outbred mothers produced their first litter by 29.5 days; for inbred females, the median was 39.5 days. For the *rhoadsi* subspecies, the median time to first reproduction was 28 days for outbred dams and 32 days for inbred dams. Although the median difference is small for the *rhoadsi* subspecies, the 75% quartile was 33 days for outbred females and 54 days for inbred ones. When the data for both subspecies were pooled, and maternal inbreeding was used as a continuous covariate, the time to first litter increased significantly as maternal inbreeding increased ($\chi_1^2 = 6.3$, $P < 0.01$). The two subspecies still differed in their responses to inbreeding ($\chi_1^2 = 15.4$, $P < 0.003$).

For the subjects of the video study, time to first litter did not differ between inbred and outbred females in either subspecies or in the pooled data for the two subspecies (Fig. 3). The trend for *P. p. rhoadsi* females, however, mirrored that of the non-video pairs, in that inbred females tended to take longer to produce their first litters than did outbred females ($\chi_1^2 = 2.76$, $P < 0.097$); no similar difference was found for *P. p. subgriseus* ($\chi_1^2 = 0.004$, $P < 0.95$).

Overall reproductive failure rates were 19.7% for *subgriseus* (14.6% for outbred females, 25% for inbred females) and 17.6% for *rhoadsi* (17.7% for outbred females, 17.5% for inbred females). If these pairs are excluded from the previous analyses, the relationships reported above still hold: inbred females that bred took longer to produce their first litters than did outbred females that bred in both populations ($\chi_1^2 = 5.33$, $P < 0.02$ for *subgriseus*, $\chi_1^2 = 9.05$, $P < 0.003$ for *rhoadsi*).

Although the two subspecies showed similar and consistent responses to maternal inbreeding, they differed in some of the specific effects of inbreeding on time to first litter birth. For both the *rhoadsi* and *subgriseus* subspecies, the rate and probability of producing a litter declined
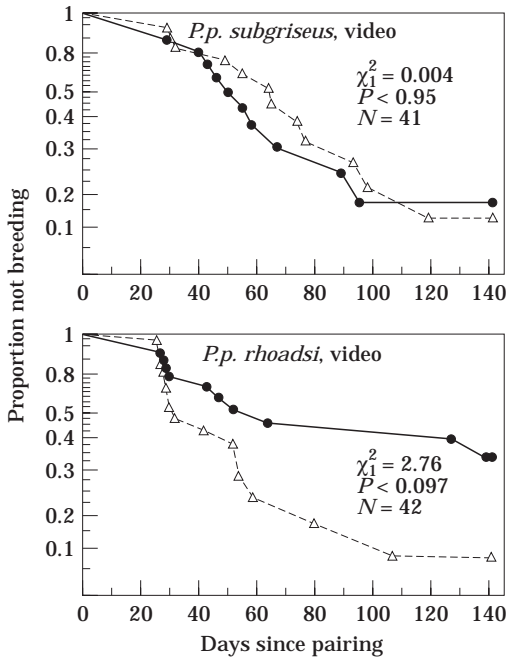
Figure 3. Survivorship curves for *subgriseus* and *rhoadsi* video study. Each graph separately plots data for inbred and outbred females. Symbols are the same as in Fig. 2.

Figure 4. Schematic survivorship curves for *subgriseus* and *rhoadsi* populations (see Fig. 2). Those parts of the curves labelled 'high' indicate a high probability of producing a live-born litter, 'very high'=greatest probability of producing a litter; 'low'=a significant decline in the probability of producing a litter, 'very low'= greatest decline in probability of producing a litter. The flat part of the curves (after approximately day 110) indicates the proportion of inbred and outbred females that were infertile.

around 40 days post-pairing. That is, females that did not produce a litter by 40 days post-pairing were extremely unlikely to do so. Outbred *rhoadsi* females had the highest probability of producing a litter before day 40, but those outbred *rhoadsi* females that had not yet produced a litter by day 40 were highly unlikely to do so after this time (Fig. 4). Inbred *rhoadsi* females, and outbred *subgriseus* females, had a high probability of producing a litter prior to day 40. Inbred *subgriseus* females had the lowest reproductive rate during this time. After day 40, all remaining *subgriseus* females and inbred *rhoadsi* females showed a comparable and substantial decline in probability of producing a litter.

For the *subgriseus* subspecies, inbred and outbred females did not differ in their probability of producing a litter after day 40. On the other hand, *rhoadsi* pairs with outbred females were highly unlikely to produce a litter if they had not done so by 40 days post-pairing. Only 13 outbred *rhoadsi* females had not yet produced a litter by 40 days post-pairing, but of the 13, only two (15%) went
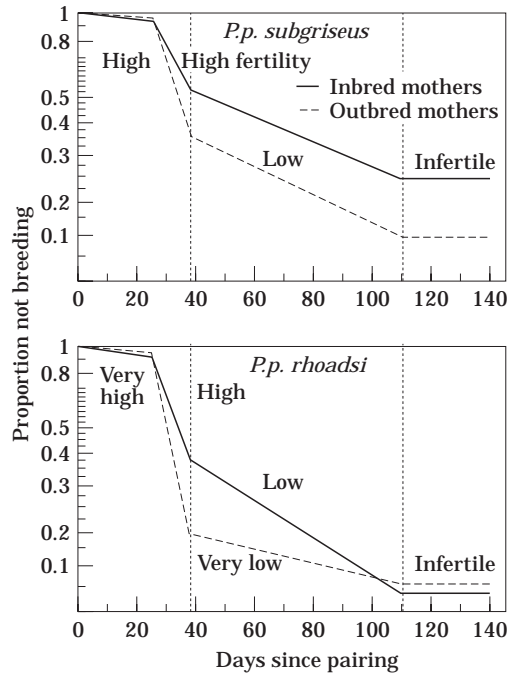
on to produce a litter. In contrast, 25 pairs with inbred females had not yet produced a litter by 40 days post-pairing, and 14 of these (56%) produced a litter by the end of the study period (120 days post-pairing). A *G*-test on the number of inbred and outbred females producing a litter before versus after 40 days indicated that outbred *rhoadsi* females were more likely to produce a litter before 40 days than were inbred females, and were highly unlikely to produce a litter if they had not done so by day 40 ($G_3=9.12$, $P<0.05$). These patterns reflect not only the stochastic nature of conceiving on a given cycle, but also the differential impact of inbreeding on the two subspecies.

Neither inbreeding of sire nor of litter was consistently predictive of time to first litter (*subgriseus*: Wilcoxon $\chi^2_1=0.008$, $P<0.93$ for sire,
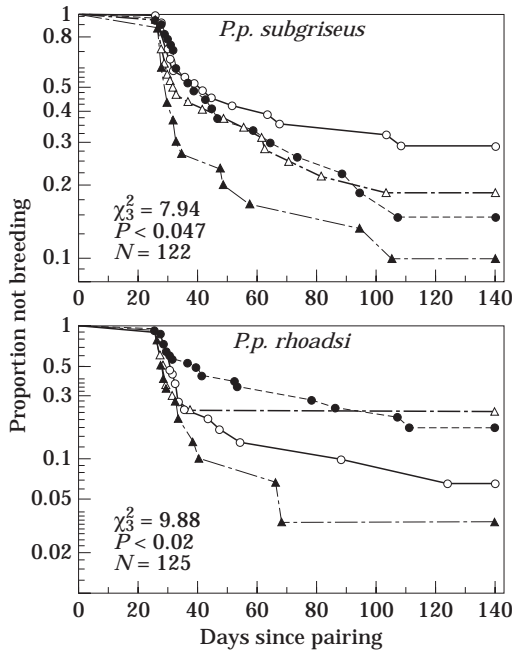
Figure 5. Survivorship curves for time to first litter for *subgriseus* and *rhoadsi* populations, separating inbred dams that would produce inbred litters (○) from inbred dams that would produce outbred litters (●), and outbred dams that would produce inbred litters (▲) from outbred dams that would produce outbred litters (△).

$\chi_1^2=0.87$, $P<0.35$ for litter; *rhoadsi*: $\chi_1^2=1.88$, $P<0.17$ for sire, $\chi_1^2=0.23$, $P<0.63$ for litter). Figure 5 presents survivorship curves based on inbreeding coefficient of the female, and what the inbreeding coefficient of the litter would be, if a litter were produced. The two subspecies yielded different results. For the *subgriseus* subspecies, inbred females that would produce inbred litters (i.e. inbred females that are related to their mates) took the longest to start breeding; in fact, 29% of pairs in which the female was inbred and related to her mate failed to reproduce. At the opposite extreme, outbred females with inbred litters (outbred females that are related to their mates) were quickest to begin breeding, and only 10% of pairs in this category failed to breed. For the *rhoadsi* subspecies, inbred females that would produce outbred litters (inbred females that are not related to their mates) were slowest to begin breeding, with 23% of pairs in this category failing to breed and a median time to first litter

of 39 days. Inbred females that would produce inbred litters were considerably faster; only 12.5% of these pairs failed to breed, and their median time to first litter was 30 days. Outbred females did not differ in their median time to first litter based on whether they were related to their mates (median time to first litter of 28 days for outbred females that were related to their mates and 29 days for outbred females that were not related to their mates). Almost 30% of outbred females that would produce outbred litters failed to breed, however, but only 7% of outbred females that were related to their mates did not reproduce.

Inbreeding and Behaviour

Subgriseus females that were inbred were significantly less active (i.e. spent at least 40% of the nocturnal time inactive) than were outbred females (logistic regression, Wald $\chi_1^2=4.27$, $P<0.04$). A female with an inbreeding coefficient of 0.5, for example, would be expected to be inactive 44% of the nocturnal period, whereas a fully outbred female ($f=0$) would be inactive only 4% of the time. No difference was seen for the *rhoadsi* females (Wald $\chi_1^2=0.00$, $P<0.998$). Using logistic regression, inbred *rhoadsi* males, but not *subgriseus* males, were significantly less active than were outbred males (Wald $\chi_1^2=4.35$, $P<0.04$ for *rhoadsi*; $\chi_1^2=0.19$, $P<0.67$ for *subgriseus*). The difference is small in magnitude, however, and due largely to a few inactive, inbred *rhoadsi* males (Fig. 6). The effect of inbreeding on activity was most pronounced for animals with the highest inbreeding coefficients (>0.3; an animal that was the product of a full-sibling mating would have an inbreeding coefficient of 0.25). Females with inbreeding coefficients greater than 0.25 were twice as likely to be inactive as females with lower inbreeding coefficients. For the video study, for which the animals were in general less active, no significant differences were found for any of the behaviours observed based on inbreeding coefficient of male, female or litter.

DISCUSSION

Activity level of females during the first 25 days after pairing predicted future reproductive success in our main study groups; pairs in which the
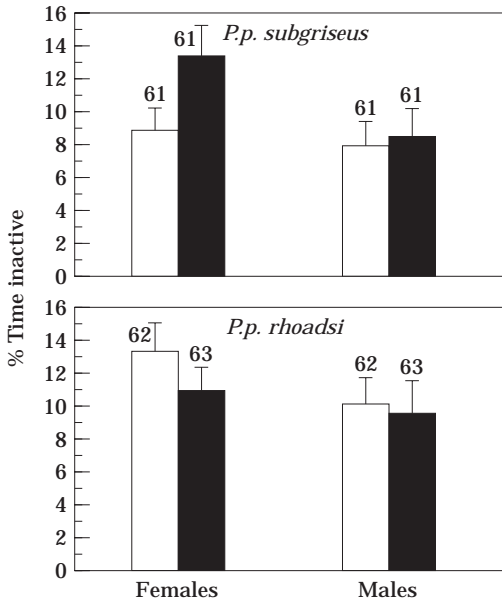
Figure 6. Mean inactivity level for inbred (■) and outbred (□) *P. p. subgriseus* (top) and *P. p. rhoadsi* (bottom). Error bars are standard errors.

females remained more inactive during the nocturnal period were less likely to breed than pairs in which the female remained active. The observed pattern in levels of inactivity, assessed during the first weeks after pairing, was thus associated with a consistent difference in reproductive performance as much as 3 to 4 months later; less active females dropped farther behind in reproductive success with time. The duration of our study (4 months after pairing) approximates, and probably somewhat exceeds, the average life expectancy of *P. polionotus* in the wild (Smith 1966; N. Holler, unpublished data; J. Layne, unpublished data). Thus, not only does a delay in reproduction represent a potentially significant loss of fitness, but it may also often result in a complete loss of lifetime reproductive opportunity. Activity level during the first weeks after pairing may in fact predict lifetime reproductive success in this small, monogamous rodent.

Behaviour influences reproductive success both directly, via parent–offspring interactions, and indirectly, via intra-pair interactions. This latter route may be especially significant in species that maintain a long-term pair bond (Alberts & Gubernick 1990). Mate-switching has been shown

to lower lifetime reproductive success in the monogamous California mouse, *P. californicus* (Ribble 1992). Gubernick (1994) suggested that the role of the pair bond has not received enough attention in the context of mating and parental care systems.

The finding of this basic relationship between behaviour shortly after pairing and lifetime reproductive success was strengthened considerably by findings in two additional data sets that extend the range of inactivity. First, the pairs used in the videotape study, which were less active, were also slower to begin breeding and less likely to breed at all than were other pairs. The lighting regime approximated the light level that these animals would experience on a fully-moonlit night, and *P. polionotus* reduce activity levels on moonlit nights (Wolfe & Tan Summerlin 1989). Second, the small pilot study on *P. p. leucocephalus* indicated that the extremely high level of inactivity for females of this subspecies during the nocturnal period (over twice that observed for those *subgriseus* and *rhoadsi* pairs that failed to breed) was associated with extremely poor reproduction (<7% of pairs reproduced). This finding further supports the conclusion that inactivity is an indicator of a delay in time to first reproduction and of reproductive failure. These additional study groups (with the exception of the *rhoadsi* videotaped subjects) fell well above the line of predicted probability of reproductive failure in Fig. 1. Thus, very high levels of inactivity were associated with even greater risk of reproductive failure than predicted from the more limited variability in our main data set.

Why inactivity should be associated with decreased reproductive success is unclear. *Peromyscus* females in oestrus increase their activity levels (Falls 1968). Smith (1966) observed substantial increases in the activity of mating pairs of *P. polionotus* compared to other pairs. Thus, the hormonal milieu may differ for females that breed and females that fail to produce litters, possibly leading to changes in behaviour. One possible explanation is that females in pairs that fail to breed or are slow to begin breeding are acyclic or cycling irregularly.

Preliminary findings concerning cyclicity in these subspecies suggest that inbred females have longer cycles and are more prone to pseudopregnancy than are outbred females (K. Koeninger, L. Fedderson & S. Margulis, unpublished data).

Outbred females were more likely to have a pattern of regular cycles, with an occasional anovulatory cycle, than were inbred females. Longer intervals between fertile periods would lead to an overall lower conception rate for inbred females when compared to outbred females. The sparse data on natural populations suggest that it may be advantageous to produce a litter as soon as possible after pairing, because persistence times on trapping grids have been estimated to average from two to four months (Rave & Holler 1992; J. Layne, unpublished data; N. Holler, unpublished data) and the population turnover rate is believed to be extremely high (Smith 1966). Thus, any extended delay in the onset of reproduction might drastically increase the probability of reproductive failure.

The observed difference between the *P. p. subgriseus* and *P. p. rhoadsi* females in the video study represents another way in which inbreeding affected the two subspecies differently. *Subgriseus* females appeared to be more sensitive to the effects of continuous red light than were *rhoadsi* females. Outbred *subgriseus* females were virtually identical to inbred females with respect to time to first litter and probability of breeding. In contrast, the observed trend for outbred and inbred *rhoadsi* females was identical to that seen in outbred and inbred females that were not exposed to direct red light. The videotaped *rhoadsi* females fell on the line of predicted probability of reproductive failure for their level of inactivity; the *subgriseus* females fell well above the line.

We can only speculate about possible mechanisms by which *Peromyscus* may determine their relationship to their prospective mates, and possibly use this relationship to assess the suitability of the partner as a mate. In nature, individual animals potentially have the opportunity to choose a mate, but in the present study individuals were presented with a single subject with which they could breed, or not, but they could not demonstrate their own mate choice. Odour cues are commonly used for communication among rodents (King 1968). Mate choice may be based on a variety of factors, including genetic relatedness (presumably based on familiarity: D'Udine & Alleva 1983; Dewsbury 1988) and sharing of specific alleles at the major histocompatibility complex (MHC; Potts et al. 1991). *Mus musculus* (as well as rats and possibly humans) can distinguish different MHC haplotypes based on urine odour (Yamazaki et al. 1976; Beauchamp et al. 1985; Yamazaki et al. 1988), which might form the basis of a mate-choice system. Potts et al. (1991) found that *M. musculus* appear to avoid mating with individuals with which they share MHC alleles. Sharing of MHC alleles has been linked to decreases in reproductive success in humans (Ober et al. 1988, 1992). A mate-choice study in *P. polionotus* may serve to elucidate possible mechanisms involved in the relationship between inbreeding and reproductive success.

For the *subgriseus* subspecies, inbreeding coefficient of the female was a significant predictor of activity level, with activity level decreasing as inbreeding coefficient increased. This effect was primarily due to high levels of inactivity at the highest inbreeding levels ($f > 0.3$). This behavioural difference mirrored the difference in reproductive timing: inbred females were slower to breed and less likely to breed, and females that were less active were less likely to breed. Inbreeding coefficient was a significant predictor of activity level for *rhoadsi* males, but not *subgriseus* males, but in neither subspecies did male inbreeding affect reproductive success. Thus pairs in which males were inbred did not experience decreased reproductive success, despite the effect of inbreeding on male behaviour. Although our data support a strong relationship between behaviour and reproductive success, and between maternal inbreeding and reproductive success, the association between inbreeding and behaviour is less consistent. Inbreeding-associated behavioural changes may have a greater impact on parental than on pairing behaviour (Margulis 1996).

Of the three components of this study, maternal inbreeding, behaviour, and reproductive success (Fig. 7), inbreeding has a much greater effect on both behaviour and reproductive success in the *subgriseus* subspecies. Inbred females fell above the line of expected probability of reproductive failure for a given level of inactivity; outbred females did not. The *subgriseus* pairs from the video study showed similar and substantial declines in reproductive success regardless of maternal inbreeding, but the inbred group showed a much greater increase in level of inactivity. Videotaping caused an overall reduction in activity level for both subspecies, but did not cause the expected decline in reproductive success for the outbred *rhoadsi* pairs. The *rhoadsi* subspecies appeared to be less sensitive to the effects
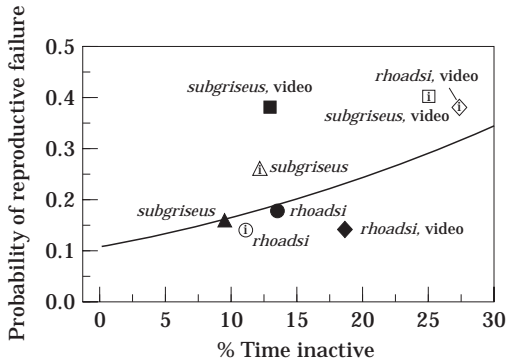
Figure 7. Predicted and observed probability of reproductive failure based on mean level of inactivity and maternal inbreeding. Maternal inbreeding was dichotomized: inbred=f>0.1, outbred=f<0.1. Both main and video study groups are plotted. Inbred subjects are denoted by an 'i' in the symbol. Solid symbols represent outbred subjects.

of inbreeding and inactivity than did the *subgriseus* subjects. These different sensitivities to inbreeding, and to exposure to constant red light (in the videotape study), were not attributable to known biological differences, and may be attributable to founder effect or drift, processes that have been shown to be important in the manifestation of inbreeding depression in these subspecies (Lacy et al. 1996).

Inbreeding depression can manifest itself in adult animals. The specific traits that are affected may vary, however, due largely to founder effect. This study has several implications both for captive populations and for small, isolated wild populations. First, inbreeding can affect behavioural as well as morphological traits. This relationship has not been well-studied and may contribute to inbreeding depression. Second, by ignoring the role of inbreeding in the adult animal, we may be greatly underestimating the extent of inbreeding depression in captive populations. Finally, because the particular traits that are affected by inbreeding are determined largely by chance events, it will prove difficult to predict how inbreeding will affect different species and different populations of the same species. Regardless of the traits that are affected, a depression in reproductive success as a consequence of inbreeding in adult animals may be a likely outcome. The possible detrimental effects of inbreeding on adult behaviour may be substantial: we may be grossly underestimating the extent of inbreeding depression by not measuring its effects on behaviour, by considering only effects on juvenile viability and by ignoring effects on adult fertility and reproductive behaviour.

REFERENCES

Alberts, J. R. & Gubernick, D. J. 1990. Functional organization of dyadic and triadic parent–offspring systems. In: *Mammalian Parenting* (Ed. by N. A. Krasnegor & R. S. Bridges), pp. 416–440. New York: Oxford University Press.

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.

Beauchamp, G. K., Yamazaki, K., Wysocki, C. J., Slotnick, B. M., Thomas, L. & Boyse, E. A. 1985. Chemosensory recognition of mouse major histocompatibility types by another species. *Proc. natn. Acad. Sci. U.S.A.*, **82**, 4186–4188.

Brewer, B. A., Lacy, R. C., Foster, M. L. & Alaks, G. 1990. Inbreeding depression in insular and central populations of Peromyscus mice. *J. Hered.*, **8**, 257–266.

Dewsbury, D. A. 1988. Kin discrimination and reproductive behavior in muroid rodents. *Behav. Genet.*, **18**, 525–536.

Falconer, D. S. 1989. *Introduction to Quantitative Genetics.* 3rd edn. New York: John Wiley & Sons.

Falls, J. B. 1968. Activity. In: *Biology of* Peromyscus *(Rodentia)* (Ed. by J. A. King), pp. 543–570. Stillwater, Oklahoma: American Society of Mammalogists.

Foltz, D. W. 1979. Genetics and mating system of the oldfield mouse (*Peromyscus polionotus*). Ph.D. thesis, University of Michigan.

Foltz, D. W. 1981. Genetic evidence for long-term monogamy in a small rodent, *Peromyscus polionotus. Am. Nat.*, **117**, 665–675.

Gubernick, D. J. 1994. Biparental care and male-female relations in mammals. In: *Infanticide and Biparental Care* (Ed. by S. Parmigiani & F. S. vom Saal), pp. 427–463. New York: Harwood Academic Publishers.

Hosmer, D. W., Jr & Lemeshow, S. 1989. *Applied Logistic Regression.* New York: John Wiley & Sons.

Kalbfleisch, J. D. & Prentice, R. L. 1980. *The Statistical Analysis of Failure Time Data.* New York: John Wiley & Sons.

King, J. A. 1968. Psychology. In: *Biology of* Peromyscus *(Rodentia)* (Ed. by J. A. King), pp. 496–542. Stillwater, Oklahoma: American Society of Mammalogists.

Kirkpatrick, M. & Lande, R. 1989. The evolution of maternal characters. *Evolution*, **43**, 485–503.

Lacy, R. C. 1992. The effects of inbreeding on isolated populations: are minimum viable population sizes predictable? In: *Conservation Biology: the Theory and Practice of Nature Conservation, Preservation, and Management* (Ed. by P. L. Fiedler & S. H. Jain), pp. 277–296. New York: Chapman & Hall.

Lacy, R. C., Alaks, G. & Walsh, A. 1996. Hierarchical analysis of inbreeding depression in *Peromyscus polionotus. Evolution*, **50**, 2187–2200.

Lynch, C. B. 1977. Inbreeding effects upon animals derived from a wild population of *Mus musculus. Evolution*, **31**, 526–537.

Lynch, C. B. & Possidente, B. P., Jr. 1978. Relationships of maternal nesting to thermoregulatory nesting in house mice (*Mus musculus*). *Anim. Behav.*, **26**, 1136–1143.

Margulis, S. W. 1996. The effects of inbreeding on parental behavior and reproductive success in two subspecies of the oldfield mouse, *Peromyscus polionotus.* Ph.D. thesis, The University of Chicago.

Ober, C., Elias, S., O'Brien, E., Kostyu, D. D., Hauck, W. W. & Bombard, A. 1988. HLA sharing and fertility in Hutterite couples: evidence for prenatal selection against compatible fetuses. *Am. J. Reprod. Immunol. Microbiol.*, **18**, 111–115.

Ober, C., Elias, S., Kostyu, D. D. & Hauck, W. W. 1992. Decreased fecundability in Hutterite couples sharing HLA-DR. *Am. J. hum. Genet.*, **50**, 6–14.

Packer, C. & Pusey, A. E. 1993. Dispersal, kinship, and inbreeding in African lions. In: *The Natural History of Inbreeding and Outbreeding* (Ed. by N. Wilmsen Thornhill), pp. 375–391. Chicago: The University of Chicago Press.

Potts, W. K., Manning, C. J. & Wakeland, E. K. 1991. Mating patterns in seminatural populations of mice influenced by MHC genotype. *Nature, Lond.*, **352**, 619–621.

Ralls, K. & Ballou, J. 1982a. Effects of inbreeding on infant mortality in captive primates. *Int. 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.

Rave, E. H. & Holler, N. R. 1992. Population dynamics of beach mice (*Peromyscus polionotus ammobates*) in southern Alabama. *J. Mammal.*, **73**, 347–355.

Ribble, D. O. 1992. Lifetime reproductive success and its correlates in the monogamous rodent, *Peromyscus californicus. J. Anim. Ecol.*, **61**, 457–468.

SAS Institute. 1988a. The LIFETEST Procedure. *SAS Technical Report P-179* Release 6.03, pp. 49–88.

SAS Institute. 1988b. The NPAR1WAY Procedure. *SAS/STAT User's Guide*, Release 6.03, pp. 713–726.

SAS Institute. 1990. The LOGISTIC Procedure. *SAS Technical Report P-200*, Release 6.04, pp. 175–230.

Smith, M. H. 1966. The evolutionary significance of certain behavioral, physiological, and morphological adaptations of the old-field mouse, *Peromyscus polionotus.* Ph.D. thesis, University of Florida.

D'Udine, B. & Alleva, E. 1983. Early experience and sexual preferences in rodents. In: *Mate Choice* (Ed. by P. Bateson), pp. 311–327. Cambridge: Cambridge University Press.

Wolfe, J. L. & Tan Summerlin, C. 1989. The influence of lunar light on nocturnal activity of the old-field mouse. *Anim. Behav.*, **37**, 410–414.

Yamazaki, K., Boyse, E. A., Mike, V., Thaler, H. T., Mathieson, B. J., Abbott, J., Boyse, J., Zayas, Z. A. & Thomas, L. 1976. Control of mating preferences in mice by genes in the major histocompatibility complex. *J. exp. Med.*, **144**, 1324–1335.

Yamazaki, K., Beauchamp, G. K., Kupniewski, D., Bard, J., Thomas, L. & Boyse, E. A. 1988. Familial imprinting determines H-2 selective mating preferences. *Science*, **240**, 1331–1332.