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DIFFERENTIAL EFFECTS OF INBREEDING AT JUVENILE AND ADULT LIFE-HISTORY STAGES IN *PEROMYSCUS POLIONOTUS*

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Inbreeding depression traditionally has been measured at early life-history stages, such as neonatal or juvenile viability or deficits in growth and development. I investigated additional fitness components by evaluating impact of inbreeding on reproductive success of adult females in a captive colony of oldfield mice (*Peromyscus polionotus subgriseus* and *P. p. rhoadsi*). By first conducting analyses on only inbreeding of litters (i.e., relatedness of parents) and then adding maternal effects (mother's inbreeding coefficient independent of parental relatedness, parity, and maternal inactivity), I illustrated effect of inbreeding on life-history characters that were manifested in adulthood. Young in inbred litters ($f > 0.1$) tended to be smaller than young in outbred litters, but inbred litters did not exhibit any decrements in survival probability. Number of young raised to weaning from inbred and outbred litters did not differ. Inbred females (inbreeding coefficient $f > 0.1$) reared significantly fewer young than did outbred females ($f < 0.1$) (independent of whether parents were related and therefore independent of whether young themselves were inbred). Differences between the two subspecies were found for a number of fitness traits. These differences are attributed to chance differences in genetic makeup of founder stocks (founder effect). Examining inbreeding effects only at a single life-history stage (i.e., during the juvenile period) and only on a single fitness trait (juvenile viability) may result in serious underestimation of the extent of inbreeding depression.

Key words: *Peromyscus polionotus*, oldfield mouse, inbreeding depression, life-history stage

An almost universal approach to the study of inbreeding depression in non-domesticated animals has been to examine inbreeding effects on offspring viability. Seminal studies (Ballou and Ralls, 1982; Ralls and Ballou, 1982a; 1982b; Ralls et al., 1979, 1988) clearly documented widespread occurrence of inbreeding depression in captive mammalian populations but focused on juvenile mortality as their measure of inbreeding depression. The implicit assumption of such studies thus has been that an inbred individual that survived to breeding age did not suffer from an inbreeding depression. In fact, an inbred adult may have suffered a severe inbreeding depression if it failed to breed, produced poor-quality offspring, or failed to raise its offspring.

In those few studies that have considered inbreeding effects on fertility, number of surviving offspring has been measured, which thereby confounded deficiencies of offspring with possible parental ones (except for Packer and Pusey, 1993). That is, in those cases where offspring were inbred (i.e., parents were related to one another), parents also were often inbred, or the level of inbreeding of the parents was not controlled (except for Brewer et al., 1990). That offspring survival is dependent on both offspring phenotypes and parental phenotypes has been incorporated only recently into genetic models of populations, where research has typically focused on consequences of fitness of genotypes of only a single generation (Kirkpatrick and Lande, 1989).

Possible consequences of inbreeding at later life-history stages and on subsequent reproductive success largely have been ignored (Hedrick and Miller, 1992). This stems in part from difficulty in accurately identifying and the labor-intensive nature of quantifying variables that measure effects of inbreeding on reproductive success. It is clear, however, that measuring effects of inbreeding at later life-history stages is important. In their review of inbreeding depression, Charlesworth and Charlesworth (1987) pointed out that experimental evidence from *Drosophila* suggested that inbreeding may have greater effects on reproduction than on viability. Inbreeding has been shown to decrease mating ability of males in *Drosophila melanogaster* (Miller and Hedrick, 1993; Sharp, 1984). Maternal activity patterns in oldfield mice (*Peromyscus polionotus*) significantly affected reproductive success and in turn were influenced by maternal inbreeding (Margulis, 1996; Margulis and Altmann, 1997).

By ignoring possible effects of inbreeding on adults, we may underestimate greatly the extent of inbreeding depression. This is an especially serious problem in captive populations and small remnant wild populations, where inbreeding is often unavoidable. Such populations are often hampered by poor reproductive success or failure of pairs to breed (Baker et al., 1996; Estep and Dewsbury, 1996; Lacy et al., 1993; Wildt, 1996). Such problems may represent hidden forms of inbreeding depression, i.e., inbreeding depression at an adult stage in a life history. Furthermore, the way in which inbreeding manifests itself and the particular traits that are affected by inbreeding are variable, making predictability of inbreeding effects problematic. Considerable research on *P. polionotus* points to chance effects as the best explanation for the variation in the traits upon which inbreeding depression acts (Brewer et al., 1990; Lacy et al., 1996). Captive populations often are started with a small number of individuals and endangered wild populations may ex-

perience population bottlenecks or fragmentation of range; situations that could leave populations highly susceptible to chance founder effects.

I examined how inclusion of maternal traits (maternal inbreeding, parity, level of inactivity) altered interpretation of the magnitude and scope of inbreeding depression. First, I took the common approach of measuring survival of offspring as the indicator of inbreeding depression; i.e., if inbred litters contained fewer young and exhibited reduced viability or reductions in developmental traits compared to outbred litters. Second, I added another dimension to my analysis of inbreeding depression by examining its possible effect on adults. I predicted that results of these two analyses would differ, based on the contribution of maternal inbreeding and related maternal traits. In addition, although it is impossible to fully assess ecological and evolutionary differences between *P. p. rhoadsi* and *P. p. subgriseus*, there do not appear to be any obvious a priori genetic or ecological reasons for these two subspecies to differ in biologically meaningful ways with respect to inbreeding. Any differences between the two subspecies therefore may be attributed to founder effect, as was the conclusion of Lacy et al. (1996).

MATERIALS AND METHODS

Subjects.—Two subspecies of the oldfield mouse, *Peromyscus polionotus*, were subjects of this research. Specimens of *P. p. subgriseus* were taken from the Ocala National Forest in north-central Florida in 1990; *P. p. rhoadsi* was captured near Lake Placid, Florida, in 1990 and 1991 (Lacy et al., 1996). Captive stocks of both subspecies were maintained at Brookfield Zoo and have been the subject of intensive genetic study (Brewer et al., 1990; Lacy, 1992; Lacy et al., 1996). Subjects of my study were 6th to 8th generation captive-born mice of known pedigree. Extensive pedigrees of the captive population allowed me to establish experimental pairings with known levels of inbreeding. Inbreeding coefficients (f) were calculated under the assumption that wild-caught founders of captive

TABLE 1.—*Experimental design and sample sizes for Peromyscus polionotus subgriseus* ($N = 124$) and *P. p. rhoadsi* ($N = 125$) in the present study. All subjects were at least 55 days of age at the time of pairing, and pairs remained together through the rearing of three litters, or for 120 days, whichever came first ($i =$ inbred, range of inbreeding coefficient, $f = 0.1010$ – 0.5859), $o =$ outbred, range of $f = 0$ – 0.0980).

Maternal inbreeding condition	Paternal inbreeding condition	Litter inbreeding condition	N	
			<i>P. p. subgriseus</i>	<i>P. p. rhoadsi</i>
i	i	i	16	16
i	i	o	14	17
o	o	i	15	15
o	o	o	16	17
o	i	i	16	16
o	i	o	15	14
i	o	i	16	15
i	o	o	16	15

stocks were neither inbred nor related. Details of the captive breeding protocols are available in Lacy (1992) and Lacy et al. (1996). Because the primary research goal of the mouse colony of the Brookfield Zoo has been examination of inbreeding effects, pairings were designed to produce a wide range of inbreeding levels in each generation (including non-inbred litters produced by pairing mice descended from distinct sets of founders). Preliminary analyses indicated that some purging of deleterious alleles (loss of deleterious alleles as a result of inbreeding and selection) occurred in inbred *P. p. rhoadsi* (R. C. Lacy and J. Ballou, in litt.), but no such amelioration of effects of inbreeding was seen after many generations of inbreeding in *P. p. subgriseus*. Thus, some effects of inbreeding reported here may have been reduced relative to inbreeding depression that would be manifest in wild populations that had no prior history of inbreeding.

Experimental design and data collection.—The study group consisted of 124 *P. p. subgriseus* pairs and 125 *P. p. rhoadsi* pairs, divided into eight experimental groups based on maternal inbreeding, paternal inbreeding, and inbreeding coefficient of future litters, if produced (Table 1). For the purposes of study design, animals with inbreeding coefficients (f) >0.1 were considered inbred; animals with $f < 0.1$ were con-

sidered outbred. The cut-off of 0.1 was chosen for several reasons. First, low levels of inbreeding are impossible to avoid even in a large outbreeding population. In addition, inclusion of individuals with very low coefficients of inbreeding, as has often been done in the past (Ballou and Ralls, 1982; Ralls and Ballou, 1982a, 1982b; Ralls et al., 1979), confounds the inbreeding effect with the effect of captivity. This scheme allowed me to separate effects of inbreeding on the parent from inbreeding on the litter. All observations were made without observer knowledge of inbreeding level of subjects or the relationship of pair members to one another.

Peromyscus polionotus is monogamous and nocturnal (Foltz, 1979, 1981). 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. All subjects were ≥ 55 days of age at the time of pairing. Pairs were kept together through birth and rearing of three litters, or for 120 days if the pair did not breed. Young were weaned at 20 days of age, ear-punched for identification, and weighed. *P. polionotus* usually undergoes a post-partum estrus; thus, females typically produce another litter within a week of the removal of a weaned litter (interlitter interval = 28.6 days ± 0.13 SE, $n = 506$ interlitter intervals). Pairs were separated at weaning of the third litter, by 120 days if no litter was present at that time, or if the pair failed to breed entirely. A pair could produce a maximum of four litters during the study.

Because previous findings (Margulis, 1996; Margulis and Altmann, 1997) indicated that the probability of producing a litter declined as level of maternal inactivity for the first few weeks after pairing increased, level of maternal inactivity was included in the analyses of probability of producing a litter. Activity level was measured with 1-h scan samples with scans at 15-min intervals (Altmann, 1974) conducted twice a week for 16 weeks during the active nocturnal period (Margulis and Altmann, 1997). Subjects were scored as being either active (locomoting about the cage) or inactive (sitting or lying immobile).

Data analysis.—Multiple linear and logistic regressions were used to analyze effects of litter inbreeding and maternal traits (maternal inbreeding, parity, inactivity) on various measures of reproductive success. Average weight of

young at 20 days of age, total litter mass at 20 days, and initial litter size were distributed normally and within-litter variance in weight of young (measured as standard deviation) was about normally distributed. These variables were analyzed using multiple linear regression (REG procedure—SAS, 1988). Within-litter variance in weight of young did not differ significantly in any of the models, so it was omitted from the analyses.

Fate of young in a litter was not independent; entire litters tended to survive or die completely. Viability was therefore measured dichotomously, with litters categorized as surviving if >50% of the young in the litter survived and as not surviving if $\leq 50\%$ of the young survived (Lacy et al., 1996). Similarly, probability of a pair producing a litter and a pair that produced a litter producing a subsequent litter were categorized dichotomously. Probability of producing a first litter differed significantly in some of the models, but probability of producing subsequent litters did not. The latter was omitted from the analyses. Multiple logistic regression was used to assess effect of continuous or categorical predictor variables on dichotomous response variables (Hosmer and Lemeshow, 1989; LOGISTIC procedure—SAS, 1990). Because the logistic regression model was not linear, regression coefficients were not as readily interpretable as linear-regression coefficients. For clarity, results included not only logistic-regression coefficients but a calculation of the instantaneous slope, which can be interpreted like a linear-regression coefficient.

Overall reproductive success was measured in two ways. First, the mean number of surviving young per female produced by 120 days post-pairing that survived to weaning (20 days later) was calculated both for inbred and outbred females and for females that produced inbred and outbred young. These differences were assessed using Student's *t*-tests. Mean number of young produced by 30 days post-pairing that survived to weaning was used to evaluate how reproductive success changed over the 120 days of the study. Second, to estimate population-level effects of inbreeding on reproductive success, total number of surviving young produced by 120 days post-pairing by inbred and outbred females and the total number of surviving inbred and outbred young at 120 days were tabulated; differences were analyzed using χ^2 tests.

Pairs that failed to produce a litter were included only in analyses of probability of producing a first litter and total reproductive success. Only those pairs that successfully reared a litter were included in analyses of average weight of young and total litter mass. Examination of reproductive tracts of females that failed to produce litters indicated that at least six females that failed to produce litters had implantation sites, indicating that they conceived but failed to give birth to a litter. These pairs were included in analyses of initial litter size ($=0$) and viability ($=0$).

All analyses were done first considering only whether the litter was inbred (i.e., litters were the product of a mating between relatives). Analyses were then repeated, with maternal effects (maternal inbreeding coefficient, parity, and in some cases, maternal inactivity level) added to the model. Because subspecific differences were evident, data were not pooled.

RESULTS

In a similar series of analyses, Lacy et al. (1996) found that only maternal inbreeding, litter inbreeding, and parity had consistent effects on reproductive success. Other independent variables (paternal inbreeding and maternal age) and all interaction terms were either not significant or had small and variable effects. Because I used subjects derived from the same captive stocks and preliminary analyses indicated that those same trends were evident in my study, those predictor variables were excluded from analyses.

Offspring growth and viability.—Inbreeding effects were more pronounced for *P. p. subgriseus* than for *P. p. rhoadsi* (Table 2). Inbred litters contained significantly fewer young, young were significantly smaller than young in outbred litters, and total litter mass was significantly less in *P. p. subgriseus*. *P. p. rhoadsi* showed a significant difference only in total litter mass; inbred litters weighed less than outbred litters. There were no differences in the probability of a pair producing a litter when pair members were related ($=$ litter inbred) and when they were not related ($=$ litter out-

TABLE 2.—Effects of inbreeding condition of litter for various measures of reproductive performance in *Peromyscus polionotus subgriseus* and *P. p. rhoadsi*. Numbers in parentheses next to logistic regression coefficients represent an instantaneous slope, calculated as the difference in the outcome variable for $f = 0.5$ compared to $f = 0$ ($f =$ inbreeding coefficient of litter); because of the difficulty of interpreting logistic regression coefficients, these calculations provide a more readily interpretable indication of the magnitude of the observed relationship, comparable to a linear regression coefficient (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$; P values not adjusted for multiple comparisons).

Subspecies	Fitness component ^a	Adjusted mean ^b	Regression coefficients for litter inbreeding	Test statistic	<i>d.f.</i>
<i>P. p. subgriseus</i>	First litter	0.79	-0.06 (-0.01)	$\chi^2 = 0.001$	1
	Litter size	4.14	-2.17**	$F = 8.39$	1,328
	Viability	0.72	0.19 (0.02)	$\chi^2 = 0.02$	1
	Average mass of young (g)	8.74	-2.88***	$F = 16.97$	1,239
	Total litter mass (g)	36.69	-31.89***	$F = 25.04$	1,239
<i>P. p. rhoadsi</i>	First litter	0.76	2.82 (0.17)	$\chi^2 = 1.20$	1
	Litter size	4.70	-0.63	$F = 0.63$	1,379
	Viability	0.73	0.13 (0.01)	$\chi^2 = 0.013$	1
	Average mass of young (g)	7.84	0.48	$F = 0.63$	1,284
	Total litter mass (g)	37.93	-13.85*	$F = 4.56$	1,284

^a First litter = the probability of producing a first litter; litter size = number of young at discovery of a litter; viability = 0 if one-half or fewer of the young in a litter survive to weaning at 20 days, and 1 if more than one-half the young survive; average mass of young = grams at weaning; total litter mass = grams at weaning.

^b Means are adjusted for the expected mean for fully outbred litters ($f = 0$).

bred), nor did viability differ for inbred and outbred litters in either subspecies.

Mean number of surviving inbred and outbred young produced per female by 30 days and 120 days did not differ for *P. p. subgriseus* (Fig. 1a). However, significantly more inbred *rhoadsi* young survived per female (Fig. 1b) ($\chi^2 = 9.28$, *d.f.* = 1, $P < 0.005$ at 30 days; $\chi^2 = 15.87$, *d.f.* = 1, $P < 0.001$ at 120 days).

Maternal effects.—When the mother's inbreeding coefficient, parity, and level of inactivity were added to models, different and complex patterns of inbreeding effects were seen. Parity was the best predictor of initial litter size and viability in both subspecies (Table 3). The two subspecies differed significantly for most other traits. Inbred *subgriseus* females produced significantly smaller litters and their litters exhibited greater viability than litters of outbred females (Table 3). Inbred females of *P. rhoadsi* tended to be less likely than outbred females to produce a litter (Table 3). Inbred females produced heavier young than outbred females, but litter size did not

differ significantly for inbred and outbred females. In both subspecies, the probability of producing a litter was significantly greater for active females than for inactive females. Inbreeding coefficient of the litter was a significant predictor of only average weight of young and total litter mass.

Outbred *subgriseus* females tended to have more young than inbred females at 30 days, but by 120 days, inbred and outbred females did not differ in the mean numbers of surviving young that they produced (Fig. 2a). Total number of young produced by outbred females were greater than for inbred females ($\chi^2 = 7.34$, *d.f.* = 1, $P < 0.01$). Among the *rhoadsi* subspecies, inbred and outbred females did not differ in total or mean number of surviving young produced by 30 days. Mean number of young produced by 120 days tended to be greatest for outbred females, and total number of surviving young produced was significantly greater for outbred versus inbred *rhoadsi* females ($\chi^2 = 13.23$, *d.f.* = 1, $P < 0.005$, Fig. 2b).

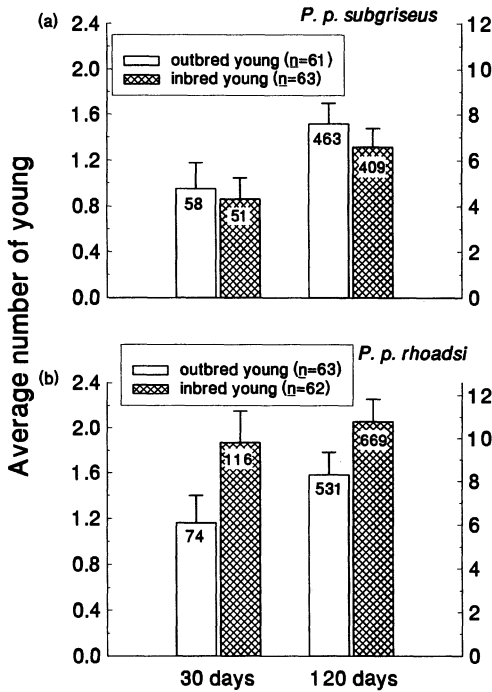


FIG. 1.—Number of outbred and inbred young produced per litter by 30- (left axis) and 120-day (right axis) post-pairings that survive to weaning, a) *Peromyscus polionotus subgriseus* and b) *P. p. rhoadsi*. *T*-tests were used to determine differences between mean number of surviving inbred and outbred young produced per female (*subgriseus*— $t = 0.31$, $d.f. = 1$, $P < 0.75$ at 30 days; $t = 0.83$, $d.f. = 1$, $P < 0.41$ at 120 days; *rhoadsi*— $t = -1.89$, $d.f. = 1$, $P < 0.06$ at 30 days; $t = -1.57$, $d.f. = 1$, $P < 0.1$ at 120 days). Total number of surviving inbred and outbred young is indicated in each bar.

DISCUSSION

Omission of maternal effects from an analysis of inbreeding depression alters the interpretation of the results. In my analysis, young produced from inbred matings were lighter, total litter mass was lower, and in some cases, litters had fewer young; however, viability was not affected. After maternal inbreeding, parity, and inactivity level were added to models, inbreeding depression (i.e., reductions in reproductive performance and success) was found to affect additional traits that have a direct bear-

ing on fitness: probability of producing a litter and litter viability. By ignoring maternal effects, most studies have underestimated the extent of inbreeding depression on traits of reproductive performance.

Differential survival of litters of inbred and outbred females might seem to contradict the lower reproductive success of inbred females. Inbred females tended to have greater levels of maternal care (Margulis, 1997), bred significantly later, and were less likely to breed than outbred females (Margulis, 1996; Margulis and Altmann, 1997). It is possible that inbred females that bred had descended from lineages that had adapted previously to inbreeding during a population bottleneck, but this is not likely to have played a major role in responses to inbreeding in my study; because inbreeding continued to have an overall detrimental effect on reproductive success. Despite increased viability of litters of inbred females (Table 3), their overall reproductive success was lower than that of outbred females. Brewer et al. (1990) and Lacy et al. (1996) did not find significantly lower inbreeding depression in stocks derived from small isolated wild populations. Thus, deleterious alleles had not been purged from these populations due to any inbreeding that occurred in the wild. However, some purging of deleterious alleles may have occurred in generations of inbreeding in captive stocks (R. C. Lacy and J. Ballou, in litt.).

There was an obvious relationship between litter size and weight of young (Tables 2 and 3); young in larger litters tended to weigh less at weaning than young in smaller litters, although total litter mass was generally not affected (total litter mass did not differ significantly for the litters of inbred and outbred females). However, it was not possible to evaluate weight of young at birth, and weight at 20 days reflected both pre- and post-natal effects on development of young. Because inbred females tended to produce fewer young per litter than did outbred females, individual young tended to be larger, and inbred females may have been

TABLE 3.—Partial regression coefficients of effects of parity, inbreeding coefficient of litter, inbreeding coefficient of mother, and maternal inactivity level for various measures of reproductive performance in *Peromyscus polionotus subgriseus* and *P. p. rhoadsi*. Fitness components and numbers in parentheses as in Table 2. P values in the last column are for the complete model (*P < 0.05, **P < 0.01, ***P < 0.0001; P values are not adjusted for multiple comparisons).

Subspecies	Fitness component	Adjusted mean ^a	Partial regression coefficients						Test statistic	d.f.	P
			Litter inbreeding	Maternal inbreeding	Parity	Maternal inactivity					
<i>P. p. subgriseus</i>	First litter	0.79	0.69 (0.05)	0.62 (0.04)	— ^b	—0.05**	(-0.53)	$\chi^2 = 14.66$	3	0.002	
	Litter size	4.27	-1.31	-1.98**	0.41***	— ^b		$F = 24.91$	3,326	0.0001	
	Viability	0.68	-0.49 (-0.05)	3.19* (0.24)	0.43*** (0.24)	— ^b		$\chi^2 = 18.11$	3	0.0001	
	Average mass of young (g)	8.44	-3.78***	3.28***	0.09	— ^b		$F = 15.03$	3,237	0.0001	
	Total litter mass (g)	36.30	-28.43***	-2.82	2.45***	— ^b		$F = 16.98$	3,237	0.0001	
<i>P. p. rhoadsi</i>	First litter	0.86	3.19 (0.17)	-3.08 (-0.23)	— ^b	-0.05**	(-0.42)	$\chi^2 = 12.78$	3	0.005	
	Litter size	4.71	-0.53	-0.16	0.44***	— ^b		$F = 14.58$	3,377	0.0001	
	Viability	0.74	0.34 (0.03)	-0.58 (-0.05)	0.29** (0.17)	— ^b		$\chi^2 = 8.29$	3	0.04	
	Average mass of young (g)	7.74	0.15	0.95*	-0.02	— ^b		$F = 1.57$	3,282	>0.05	
	Total litter mass (g)	37.31	-12.52*	1.42	2.72***	— ^b		$F = 9.90$	3,282	0.0001	

^a Means are adjusted for expected means for fully outbred litters (f = 0) born to fully outbred dams (f = 0).

^b Not applicable to this fitness component.

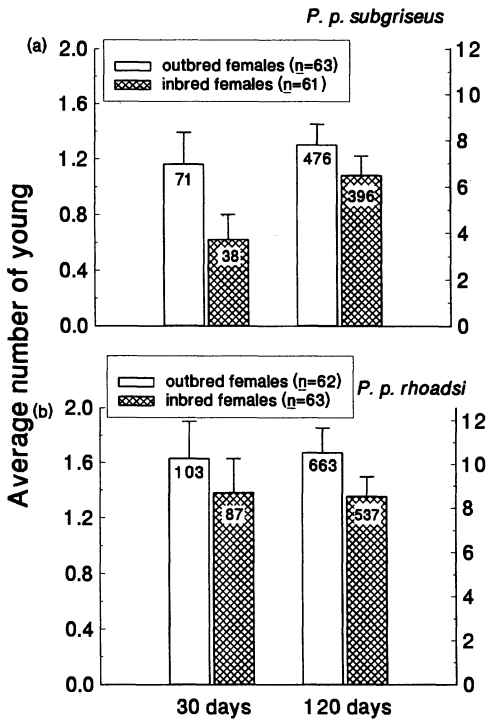


FIG. 2.—Number of young per litter produced by outbred and inbred mothers by 30- (left axis) and 120-day (right axis) post-pairings that survive to weaning, a) *Peromyscus polionotus subgriseus* and b) *P. p. rhoadsi*. *T*-tests were used to determine differences between mean number of surviving young produced per inbred and outbred female (*subgriseus*— $t = 1.89$, $d.f. = 1$, $P < 0.06$ at 30 days; $t = 0.93$, $d.f. = 1$, $P < 0.35$ at 120 days; *rhoadsi*— $t = 0.75$, $d.f. = 1$, $P < 0.45$ at 30 days; $t = 1.44$, $d.f. = 1$, $P < 0.15$ at 120 days. Total number of surviving young is indicated in each bar.

able to invest more energy (milk, thermoregulation) in each offspring.

Litters of *P. p. rhoadsi* tended to have more young than litters of *P. p. subgriseus* (Table 2), with lower average weights of young at weaning. Inbred *rhoadsi* females tended to be less likely to breed than outbred females, but that difference was not significant; nor was this pattern found for *P. p. subgriseus* (Table 3). Thus, maternal inbreeding resulted in lower reproductive success in the two subspecies, but specific traits that were affected differed. In *P. p.*

rhoadsi, inbred females were less likely to breed, but those that bred were as likely to raise their litters (i.e., viability did not differ significantly) as were outbred females (Table 3). In *P. p. subgriseus*, probability of breeding did not differ for inbred and outbred females, but inbred females produced litters with significantly fewer young (Table 3). Difference in viability (greater for inbred females) was not sufficient to counter combined effects of decreased litter size and delayed reproduction (seen in both subspecies; Margulis, 1996).

Previously, Brewer et al. (1990) found that inbreeding had variable effects on fitness traits in eight populations of *P. polionotus* and *P. leucopus*. Lacy et al. (1996) repeated this study on three subspecies of *Peromyscus* using several independent replicates, each with different founders, and obtained similar results. Variability in effects of inbreeding between random replicates of a subspecies was similar to differences between subspecies and could be traced to deleterious genes from specific founders (Lacy et al., 1996). Founder effect, a common phenomenon when populations are derived from or reduced to a small number of individuals, was the most likely explanation for those results (Lacy et al. 1996).

The overall greater effects of inbreeding in *P. p. subgriseus* could have been due to some purging in *P. p. rhoadsi*. However, subspecific differences were more a result of how traits were affected than the total extent of inbreeding depression. Thus, differences between *subgriseus* and *rhoadsi* subspecies in my study are consistent with the view that founder effect most parsimoniously explains variation in how inbreeding depression manifests itself (Lacy et al., 1996). Moreover, specific differences between subspecies were not consistent across independent replicate stocks that were measured by Lacy et al. (1996). The overall conclusion, however, is the same for both subspecies; inbred females have decreased reproductive success compared to outbred

females, independent of whether or not they are related to their mates and therefore independent of whether or not their litters are inbred. Inbreeding depression occurred in both subspecies, although it manifested itself in different traits.

My results are in general agreement with previous findings for these captive populations (Brewer et al., 1990; Lacy et al., 1996), and differences among the studies are attributed to differences in experimental design. Earlier studies on these populations controlled for maternal effects while focusing on direct effects of inbreeding in the progeny; I incorporated maternal effects into the model. Furthermore, earlier studies included data on first litters and one subsequent litter, during which only the female of this biparental-care species was present, thereby introducing confounding effects into the analyses of litter viability. However, a common pattern among mammals is for parental behavior and litter viability to be lower for first versus subsequent litters (*Microtus ochrogaster*—Wang and Nowak, 1994; *P. polionotus*—Margulis, 1996). Because pairs had 120 days in which to produce a litter and data were collected through rearing of up to four litters, with both parents present for the rearing of up to three litters, my analyses expand the existing database used by others, in which both parents were present for the rearing of the first litter only, and provide stronger support for observed patterns of inbreeding effects (both maternal and offspring) on viability.

The perspective from which inbreeding depression is evaluated may significantly influence interpretation of these findings. If a more traditional approach is taken, the *rhoadsi* subspecies may actually benefit from inbreeding, given that more inbred young survived than outbred young. This may reflect in part the purging observed in the captive *rhoadsi* population but not in the *subgriseus* population. However, if effect of inbreeding is evaluated beyond survival at weaning, inbreeding depression

was evident; inbred females had significantly lower reproductive success than did outbred females in both subspecies.

The tendency for studies of inbreeding depression to focus on traits expressed early in an organism's life is a pervasive one. However, several recent studies on widely disparate taxa have reported perhaps the first strong evidence for inbreeding depression in natural populations. Inbred land snails (*Arianta arbustorum*) had significantly higher mortality than did outbred subjects upon return to a natural garden (Chen, 1993). During episodic weather-related declines in the population, inbred song sparrows (*Melospiza melodia*) had higher mortality than outbred song sparrows (Keller et al., 1994). Among mammals, Jiménez et al. (1994) found higher mortality among inbred adult white-footed mice (*P. leucopus*) when captive-born inbred and outbred mice were released into a natural habitat. Similarly, mortality was greater for inbred common shrews (*Sorex araneus*) when survival of inbred and outbred juveniles in their natural habitat was compared (Stockley et al., 1993).

Possible effects of inbreeding on characteristics of adult fertility have begun to receive attention, but often in populations in which the extent of inbreeding depression is already known to be severe. The Florida panther (*Felis concolor coryi*), for example, has been the subject of intensive investigation in recent years (Barone et al., 1994; O'Brien, 1994; Roelke et al., 1993). The remnant population of ca. 30 Florida panthers has experienced numerous defects presumed to be genetic in origin: low sperm counts, cryptorchidism, and cardiac defects. Decreased sperm motility also has been found for inbred adult lions (*Panthera leo*) (Brown et al., 1991; Packer and Pusey, 1993; Wildt et al., 1987).

Despite recent focus on inbreeding effects on mammalian fertility, effects of inbreeding on behavioral components of fitness remains poorly investigated. This may be a potentially significant component of

inbreeding depression in species that exhibit a high degree of parental care. My investigation represents an initial attempt to incorporate parental, including behavioral, traits that bear on fitness into existing models.

Inbreeding depression can and does manifest itself in traits expressed in adult animals, but specific traits that are affected may vary. Several implications of my study for captive populations and small fragmented wild populations in which inbreeding may be unavoidable are apparent. First, by ignoring the role of inbreeding in the adult animal, we may overlook an important component of inbreeding depression in vulnerable populations. Second, behavior (i.e., level of inactivity in the present investigation) can influence reproductive performance and in turn can be influenced by inbreeding (Margulis, 1996); therefore, it is essential to monitor populations for possible behavioral deficits caused by inbreeding. Finally, because particular traits that are affected by inbreeding may be determined largely by chance events, it will prove difficult to predict how inbreeding will affect different species and even 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 appears to be a common outcome. Possible detrimental effects of inbreeding on adult reproductive performance may be substantial, and implications for captive breeding programs and small managed populations in the wild may be serious. We may be underestimating the extent of inbreeding depression by not measuring its effects on behavior, considering only early life history components of fitness, and ignoring components that are manifested in adults.

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